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THE NEW YORK ACADEMY OF SCIENCES

SECTION OF PHYSICS AND CHEMISTRY

SPECIFICITY OF ANTIBODIES*

By Fred Karush

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Introduction

The immunological properties of antibodies have presented a tantalizing challenge to physical chemists since Arrhenius[1] first applied the law of mass action to the reaction between antigen and antibody more than fifty years ago. One of the most intriguing aspects of this challenge is the high degree of specificity exhibited by the antibody molecule. The simplest manifestation of immunological specificity is to be found in the formation of soluble complexes with small molecules (haptens) carrying the antigenic group against which the antibody is directed. This interaction provides a powerful experimental tool for the study of antibody specificity. It may be employed indirectly, as has usually been the case, in the form of the hapten-inhibition technique. A fuller exploitation of the relative simplicity of the interaction is made possible by methods that distinguish between free and bound hapten, particularly the technique of equilibrium dialysis. The earliest experiments using the dialysis method were described by Marrack and Smith in 1932,[2] and were followed shortly by Haurowitz and Breinl in 1933.[3]

During the past few years we have devoted our attention to the study of the antibody-hapten interaction with emphasis on the thermodynamic features of the reaction and on the structural relationship of the components of it.[4,5] Since our studies depended on the availability of purified antibody, we have developed a fairly general and mild procedure for the specific purification of antihapten antibodies.[6] We shall omit a description of the method at this time and devote ourselves to the presentation of the results of our studies with two chemically different types of haptenic groups. These results will illustrate the kind of information that can be elicited by the experimental approach. They will also provide an informative comparison of the similarities and differences in the behavior of the two systems.

The antibody-hapten reaction has also served as a useful guide in the interpretation of experiments concerned with the structural stability of the combining region of the antibody. In particular, the effects of denaturing concentrations of urea and the splitting of disulfide bonds

*This paper, illustrated with lantern slides, was presented at a meeting of the Section on April 1, 1958. The studies reported in this article were aided by a grant from the National Science Foundation, Washington, D. C., and by Research Grant H-869 from the National Heart Institute, Public Health Service, Bethesda, Md.

on the specific reactivity of the antibody have been evaluated in this way. A description and analysis of these experiments will be found elsewhere.[7]

Antibody-Hapten Interactions

The two haptenic groups that have been primarily employed in our work are *p*-azophenyl-β-lactoside and phenyl-(*p*-azobenzoylamino)-acetate, shown in FIGURE 1. The former is designated as the Lac group

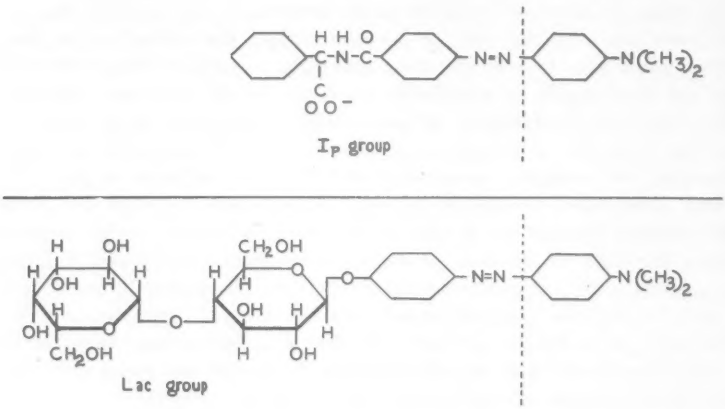


FIGURE 1. Formulas of the haptenic groups and the corresponding dyes.

and the latter, since there are two optically isomeric forms of it, is called the D-I_p group or the L-I_p group. The original observations of the antigenicity of these groups were made by Goebel *et al.*[8] and by Landsteiner and van der Scheer,[9] respectively. For the preparation of antihapten rabbit antisera these groups were coupled to bovine γ-globulin to provide the immunizing antigen. They were also reacted with dimethylaniline to give the azo dyes shown in FIGURE 1. These dyes were used in the binding experiments to be described. Their particular utility arises from their intense visible absorption bands that allow accurate concentration measurements in solutions as dilute as 10^{-5} *M*. The binding of these haptenic dyes to the homologous antibody was studied by the relatively simple procedure of equilibrium dialysis. Since only soluble complexes involving one antibody molecule per complex result from the interaction, the antibody-monohaptenic dye system has important theoretical advantages. In particular, nonspecific free energy contri-

butions arising from phase transitions and from protein-protein interactions are absent.

The same system was employed for determinations of the affinity between antibody and a variety of colorless molecules structurally similar to the homologous azohaptenic groups. Here the reduction in the binding of the azo dyes by antibody in the presence of these molecules provided the basic information from which the desired association constants were calculated with the aid of the appropriate equations.[4]

*Anti-*D-I_p *system.* We turn first to a consideration of the behavior of the anti-D-I_p system. Entirely similar results are obtained with anti-L-I_p antibody and L-I_p dye, but for the sake of simplicity and brevity we shall restrict ourselves to the isomeric system. In FIGURE 2 are shown binding results for two temperatures plotted in the form of r/c versus r, where r is the average number of dye molecules bound per antibody molecule at the concentration c of the free dye. One ad-

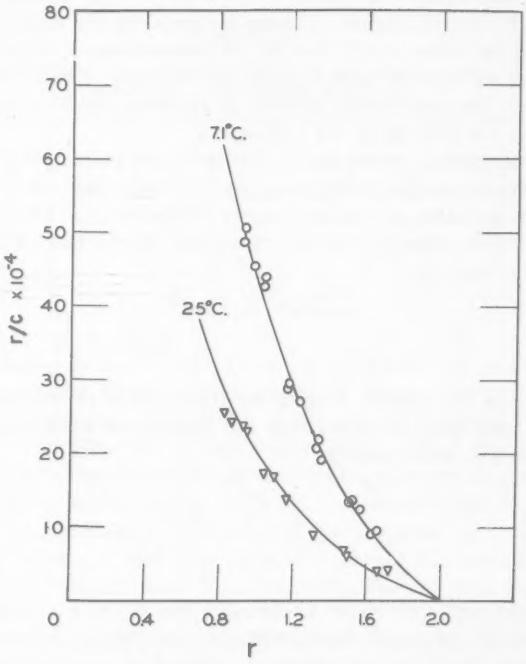


FIGURE 2. Binding results at 25° and 7.1° C., for the reaction between D-I_p dye and purified anti-D-I_p antibody. The points are experimental and the curves are theoretical. (Reprinted by permission from *The Journal of the American Chemical Society.*)[4]

vantage of this kind of plot is that extrapolation of the experimental curve to the abscissa yields a value of the antibody valence together with an appraisal of the uncertainty involved. Another useful feature is that the deviation of the curve from linearity provides a measure of the heterogeneity of the combining regions of the antibody molecules with respect to their affinity for the azohapten. If all of the antibody sites possess the same intrinsic association constant, K, the law of mass action leads to the expression

$$r/c = nK - rK \tag{1}$$

where n is the valence of the antibody.

Examination of FIGURE 2 allows us to draw a number of interesting conclusions. Since the observed values of r range from 0.8 to 1.8 a reliable extrapolation can be made. The value of 2.0 thus obtained is estimated to be accurate to within 5 per cent. Furthermore, it is evident that the antibody sites are heterogeneous, since the binding curves deviate substantially from linearity. The average value of the association constant, K, for each curve is taken as equal to the reciprocal of the value of c for which $r = 1$, that is, at half-saturation of the antibody. Finally the reaction of antibody with the azo dye is quite sensitive to temperature. The association process is characterized by a considerable decrease in the enthalpy of the system.

The heterogeneity noted above can be given quantitative expression by using the assumption, first formulated by Pauling and his associates,[10] that the distribution of the free energy of binding can be described by the Gauss error function. On this basis the distribution of association constants is given by[4]

$$\frac{dN}{N} = \frac{1}{\sigma\sqrt{\pi}} e^{-[\ln(K/K_o)]^2/\sigma^2} \, d[\ln(K/K_o)] \tag{2}$$

where K_o is an average binding constant and σ is the heterogeneity index. It can then be shown that the fraction of sites occupied as a function of c is expressed by

$$r/n = 1 - \frac{1}{\sqrt{\pi}} \int_{-\infty}^{\infty} \frac{e^{-\alpha^2}}{1 + K_o c e^{\alpha\sigma}} \, d\alpha$$

where α has been substituted for $\ln (K/K_o)/\sigma$. A value of 2 was assigned to n, and K_o, as noted above, was read from the experimental binding curve since $r/n = \frac{1}{2}$ for all values of σ when $K_o c = 1$. To obtain the most satisfactory theoretical curve numerical evaluation of the integral was carried out for several values of σ as described by Greenwood and Miller.[11] With $\sigma = 2.3$ the theoretical curves shown in FIGURE 1 are in satisfactory agreement with the experimental results.

The thermodynamic results for one preparation of anti-D-I_p antibody are shown in TABLE 1. The values of K_o for the two temperatures employed were obtained as described above and the thermodynamic functions were calculated in the usual way. It has been found for the group of six preparations that have been studied that $\Delta F°$ ranges from -6.7 to -7.8 kcal. per mole of hapten. Of particular interest is the fact that the enthalpy change ($\Delta H° = -7$ kcal.) is almost entirely responsible for the formation of the antibody-hapten complex.

A more detailed analysis of the specificity of the antibody than is provided by the above results can be made with the aid of several colorless haptens. Their affinity for the antibody was determined, as we have already pointed out, by their inhibition of binding of the azohapten. In TABLE 2 we have listed five inhibitors whose association constants have been measured for a particular antibody preparation. The affinity for D-I_p dye of this antibody at 25° C., is given by $K_o = 29.4 \times 10^4$ ($-\Delta F° = 7.48$ kcal./mole). From the association constants of the inhibitors and

TABLE 1

BINDING OF AZOHAPTENIC DYES BY HOMOLOGOUS PURIFIED ANTIHAPTEN ANTIBODY

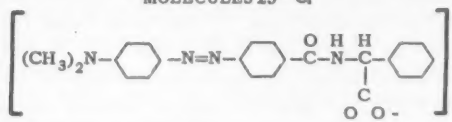
			25° C.		7.1° C.			
Dye	n	σ	$K_o \times 10^{-5}$	$-\Delta F°$ kcal./mole	$K_o \times 10^{-5}$	$-\Delta F°$ kcal./mole	$-\Delta H°$ kcal./mole	$-\Delta S°$ e.u./mole
D-I_p	2.0	2.3	2.05	7.25	4.4	7.24	7.1	0.3
Lac	2.0	1.5	1.57	7.09	4.48	7.25	9.7	-8.8

the corresponding values of $\Delta F°$ we may extract a number of interesting conclusions. In the first place we note that the D and L isomers of phenyl-(*p*-nitrobenzoylamino)-acetate differ in their effectiveness by a factor of about 200. This result implies that each of the three substituents of the asymmetric carbon atom interacts with one or more groups of the combining region of the antibody, thus giving rise to what we may call a 3-point contact. Furthermore, we note that the D isomer of the above inhibitor is bound with an affinity of only 0.5 kcal. less than that of the substantially larger D-I_p dye itself. We infer from this small difference that the energetically effective volume of the combining region of the antibody is probably not larger than that required to make contact with the entire dye molecule.

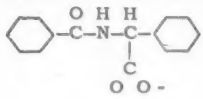
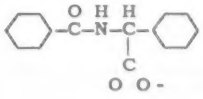
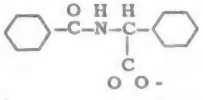
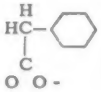
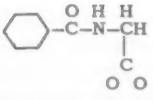
Finally, a comparison of the last three inhibitors reveals that the benzoylamino group contributes about 2 kcal. to the free energy of binding, and the phenyl group even a larger amount, 4 kcal. The energetic superiority of the phenyl group as well as the absolute value of its

TABLE 2

THE INHIBITION OF D-I_p DYE BINDING BY STRUCTURALLY RELATED MOLECULES 25° C.



Anti-D-I_p antibody + D-I_p dye, $K_o = 29.4 \times 10^4$, $-\Delta F^\circ = 7.48$ kcal./mole

Inhibitor	$K_I \times 10\text{-}4$	$-\Delta F^\circ$
D- O_2N 	12.8	6.98
L- O_2N 	0.0657	3.85
D- 	8.77	6.74
	0.285	4.72
	0.00776	2.58

contribution may appear surprising. However, it should be kept in mind that these free-energy changes are associated with the transfer of groups from an aqueous environment to the antibody site. Thus, in the case of the phenyl group, its removal from this environment allows the formation of additional hydrogen bonds between water molecules. This stabilization of the system is reflected in an increased affinity of the antibody site for the phenyl group. The lower value observed for the benzoylamino group is probably due to the loss of hydrogen bonds with water molecules that this group must undergo on combination with antibody and that is not compensated for by the formation of new hydrogen bonds.

Anti-Lac system. We may now turn to a consideration of the behavior of a different type of haptenic group, *p*-azophenyl-β-lactoside. This group differs from our first one in the two important respects that it is uncharged and that its main portion has a strong affinity for water, unlike the uncharged form of the I_p group. The presence of a disaccharide unit in the Lac group also adds interest to its antigenic properties because of its relationship to polysaccharide antigens.

Binding experiments were carried out with the Lac dye and anti-Lac antibody in the same manner as indicated for the anti-D-I_p system. The binding results for one antibody preparation are shown in FIGURE 3. It is evident that the anti-Lac system exhibits the same qualitative features as we have already seen above. Aside from the quantitative agreement as to the valence of the antibody, the experimental points do not fall on a straight line and the binding is strongly dependent on temperature.

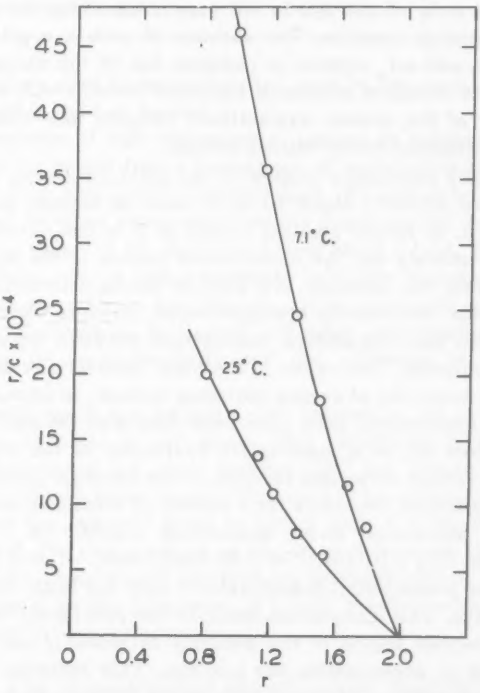


FIGURE 3. Binding results at 25° and 7.1° C., for the reaction between Lac dye and purified anti-Lac antibody. The points are experimental and the curves are theoretical. (Reprinted by permission from *The Journal of the American Chemical Society.*)[5]

Although the anti-Lac antibody is heterogeneous with respect to its affinity for the Lac dye, the heterogeneity of this system is less than that observed with the anti-D-I_p system. Application of the theoretical treatment described above to the anti-Lac results leads to quantitative agreement between the experimental points and the theoretical curves with a value of σ of 1.5. For the anti-D-I_p system, it will be recalled, a value of 2.3 for the heterogeneity index was needed.

The thermodynamic characteristics of the anti-Lac system are summarized in TABLE 1. The similarity in the values of $\Delta F°$ for the two systems is striking, particularly in view of the chemical differences between the antigenic groups. The reason for this may be, as Pauling has suggested,[12] that the requirements for the release of antibody into the circulation place an upper limit on the affinity of the antibody.

The difference in the chemical nature of the groups does evidently manifest itself in the comparative values of $\Delta S°$ and $\Delta H°$. The value of -9 e.u. per mole of Lac dye is not surprising, since we are dealing with an association reaction. The absence of such a negative entropy change in the anti-D-I_p system is probably due to the charge neutralization, with the attendant release of ion-bound water, which accompanies the formation of the anionic dye-antibody complex and which provides a positive contribution to the entropy change.

A particularly informative feature of the anti-Lac system is the large negative value of $\Delta H°$, about 10 kcal./mole of hapten, since, as we shall soon see, by far the greatest portion of it is due to the interaction between the antibody and the disaccharide portion of the hapten. Thus, it is clear that the antibody can provide strong interactions for this type of molecule, undoubtedly hydrogen bonds, which in their total effect greatly exceed the competitive interactions between water molecules and the disaccharide. The extent of the latter interactions can be judged from the high solubility of sugars, including lactose, in aqueous solution.

Inhibition experiments have also been done with the anti-Lac system and these have led to a quantitative evaluation of the energetic significance of various structural features of the haptenic group. TABLE 3 lists the association constants for a number of inhibitors measured with an antibody preparation whose association constant (K_o) for Lac dye at 25° C., was 13.4×10^4 ($-\Delta F° = 7.00$ kcal./mole). It will be observed that $-\Delta F°$ for *p*-nitrophenyl-β-lactoside is only 0.4 kcal. less than that for the Lac dye. This comparison leads to the conclusion that the energetically effective region of the antibody is probably not larger than that required to accommodate the Lac dye. This estimate agrees well with the corresponding inference made above for the anti-D-I_p antibody.

The large values of $-\Delta F°$ for methyl-β-lactoside and lactose emphasize the fact that the dominant contribution to the stability of the antibody-Lac dye complex stems from the interaction between the disaccharide portion and the protein. In view of the chemical nature of the sugar it is quite

TABLE 3

THE INHIBITION OF LAC DYE BINDING BY STRUCTURALLY RELATED MOLECULES AT 25° C.

[p-(p-DIMETHYLAMINOBENZENEAZO)-PHENYL-β-LACTOSIDE]

Anti-Lac antibody + Lac dye, $K_o = 13.4 \times 10^4$, $-\Delta F^\circ = 7.00$ kcal./mole		
Inhibitor	$K_I \times 10^{-4}$	$-\Delta F^\circ$
p-Nitrophenyl-β-lactoside	6.75	6.60
Methyl-β-lactoside	2.02	5.88
Lactose (64% β)	1.10	5.52
Cellobiose (66% β)	0.00275	1.96
Methyl-β-D-galactoside	0.00747	2.56
Methyl-α-D-galactoside	0.00134	1.54
Methyl-β-D-glucoside	0.00054	0.97

clear, therefore, that hydrogen bonding must be the main intermolecular force utilized in the Lac system.

The importance of both hexosides of lactose in determining the total affinity may be judged from a comparison of methyl-β-lactoside, methyl-β-D-galactoside and methyl-β-D-glucoside. We estimate from the ΔF° values found for these substances that the β-galactoside unit contributes about 5 kcal./mole and the β-glucoside unit about 3 kcal. Thus, although both hexosides play a major energetic role, the terminal hexoside is the dominant one. These conclusions are in agreement with the qualitative deductions drawn by Goebel and other investigators[8] from their observations on cross-reactivity and the inhibition of specific precipitation.

The specificity of the antibody with respect to the configuration of the terminal hexoside is very great. This selectivity is shown clearly by the difference of 3.6 kcal. between ΔF° for lactose and ΔF° for cellobiose, although these sugars differ only in the reversal of the hydrogen atom and the hydroxyl group on carbon atom 4 in the terminal hexoside. Finally, the configuration of the linkage between the hexosides, α or β, is also significantly involved in the specificity of the antibody, as demonstrated by the difference of 1 kcal. between methyl-β-D-galactoside and methyl-α-D-galactoside.

The Acquisition of Immunological Specificity

We now leave the firm ground of experimental fact and venture to suggest a mechanism for the acquisition of immunological specificity. In this hypothesis the disulfide bonds of the antibody molecule play a key role in accordance with experimental observations described else-

where.[7] The sequence of events leading to the appearance of antibody as we envisage them is depicted in FIGURE 4.

In agreement with a commonly held view[12] we make a sharp conceptual as well as temporal separation between the process that brings about a predetermined sequence of amino acid residues in the polypeptide chain and the process whereby the antibody molecule acquires its specificity. We believe that theories that do not make this distinction suffer

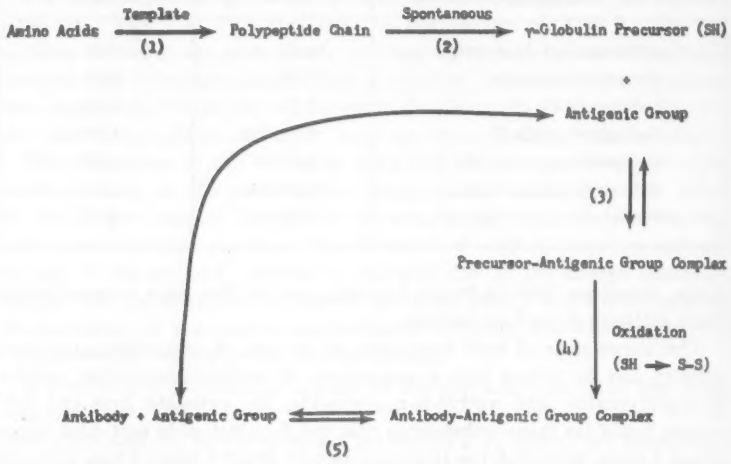


FIGURE 4. Diagram representing a mechanism for the acquisition of immunologic specificity.

from a fatal consequence of the failure to do so. This view is based on the structural argument that, if the sequence-directing template is to be able to select out the appropriate amino acid residues and allow the formation of peptide bonds, the components of the template must be in intermolecular contact with the side chain (*R* group) of each residue and the set of residues must be in a more or less extended form relative to each other. This requirement precludes any significant interaction or juxtaposition of the *R* groups themselves; that is, the template does not allow and cannot provide directly information for the development of noncovalent structure. To make the point in another way, we may say that the template can provide information only as to the sequence of amino acid residues; further development of structure depends on *R* group interactions determined or favored by the sequence itself or on the intervention of an additional component.

The view that the specificity of an antibody is associated with a unique sequence of amino acid residues is also subject to what appears to be an insurmountable difficulty. This stems from the limitations of the function of the template that we have just pointed out. In terms of

these limitations the template must be able to provide a sequence of residues such that the corresponding *R* groups will, for a particular antigenic group, subsequently interact and arrange themselves into a constellation which constitutes a structurally complementary region for that group. The essential difficulty is that there appears to be no chemically reasonable mechanism by which the template can be informed, that is, chemically or structurally altered, so as to meet this requirement. It should be emphasized that this argument applies only to the formation of proteins in the form of antibodies specific for antigenic groups foreign to the antibody-forming cell. In the case of other proteins with specific functions, for example, constitutive enzymes, we can easily imagine that evolutionary selection will have led to the development of templates that give rise to sequences of residues destined to assume structures appropriate for their specific function.

Returning to FIGURE 4, we note that, following the formation of the polypeptide chain and its release from the template, there is a spontaneous organization of the molecule in which secondary structure (helical formation) and tertiary structure are acquired. We believe that the resulting molecule, which is called the γ-globulin precursor, exists in the reduced state. The primary structural justification for this assumption relates to the nature of the template. As we have described it, the operation of the template would not allow the chain folding and juxtaposition of *R* groups which the presence or formation of disulfide bridges would require.

In addition to existing in the reduced form, and indeed because of it, the precursor is assumed to possess the rather unusual but by no means exclusive property of forming reversible complexes with a wide variety of chemical groups. This capacity for nonspecific binding we have called configurational adaptability. It is displayed most characteristically by the serum albumins, the study of which has provided what little understanding of this phenomenon we now have. It is worth noting, for example, that human serum albumin (HSA) can bind the D-I_p and the L-I_p dyes with an affinity as great as that shown by antibodies homologous to these dyes.[13] Yet the binding of these dyes by HSA takes place in the same regions of the protein molecule, whereas the antibody sites are, of course, highly discriminatory between the isomeric dyes. Such adaptability, which we also ascribe to the precursor, is attributed to the existence of regions of the protein that can assume a large number of constellations. That constellation, which is most nearly complementary to a particular antigenic group, will be stabilized by combination with this group. This selected configuration of the precursor will possess only a transient existence, however, since it will be lost on dissociation of the precursor-antigenic group complex.

If, on the other hand, the formation of intramolecular disulfide bonds occurs during the existence of the complex, we assume that the constel-

lation that has had only temporary stability now acquires an enduring rigidity independent of the presence of the antigenic group. It is not possible to state how many or which disulfides must be formed to ensure this new stability. One would infer from our experiments, however, that more than one half of the total number possible must be involved.

An interesting question that arises at this point is whether the same cysteine residues are paired with each other in different antibodies or whether different specificities involve alternative pairing. At the present time we are unable, of course, to decide between these possibilities, although the antigenic identity of antibodies would tend to favor the first one. In any case, either possibility seems to be compatible with the mechanism we have been describing. Even if the disulfide pairing is independent of specificity, we can readily see that relative movements of *R* groups and the rearrangements of nearest neighbors that occurred in the precursor would become impossible in the antibody if these changes required the separation of linked half-cystine residues by as little as a few tenths Å.

The final step in the emergence of the antibody is its dissociation from the complex in which it was born. The occurrence of this separation offers no particular difficulty since it is firmly supported by the well-established reversibility of the antigen-antibody reaction. As a result of the dissociation the antigenic group can then continue its cyclic participation in the process we have been describing.

Two final comments may be made regarding this process: (1) the conversion of sulfhydryl groups to disulfides is a reaction that can occur readily under physiological conditions and; (2) the known heterogeneity in the affinity of antibodies is readily explicable in terms of the proposed mechanism. Thus, heterogeneity would be expected if the dissociation of the precursor from the antigenic group occurred before the full number of disulfide bonds necessary to provide maximum complementarity had been formed.

References

1. ARRHENIUS, S. 1907. Immunochemistry. Macmillan. New York, N. Y.
2. MARRACK, J. & F. C. SMITH. 1932. Brit. J. Exptl. Pathol. 13: 394.
3. HAUROWITZ, F. & F. BREINL. 1933. Hoppe-Seyler's Z. physiol. Chem. 214: 111.
4. KARUSH, F. 1956. J. Am. Chem. Soc. 78: 5519.
5. KARUSH, F. 1957. J. Am. Chem. Soc. 79: 3380.
6. KARUSH, F. & R. MARKS. 1957. J. Immunol. 78: 296.
7. KARUSH, F. 1958. Serological and Biochemical Comparisons of Proteins. W. H. Cole, Ed. Rutgers Univ. Press. New Brunswick, N. J. In press.
8. GOEBEL, W. F., O. T. AVERY & F. H. BABERS. 1934. J. Exptl. Med. 60: 599.
9. LANDSTEINER, K. & J. VAN DER SCHEER. 1928. J. Exptl. Med. 48: 315.
10. PAULING, L., D. PRESSMAN & A. L. GROSSBERG. 1944. J. Am. Chem. Soc. 66: 784.
11. GREENWOOD, R. E. & J. J. MILLER. 1948. Bull. Am. Math. Soc. 54: 765.
12. PAULING, L. 1940. J. Am. Chem. Soc. 62: 2643.
13. KARUSH, F. 1954. J. Am. Chem. Soc. 76: 5536.

SECTION OF GEOLOGY AND MINERALOGY

SAMPLE CONTAMINATION AND RELIABILITY OF RADIOCARBON DATES*

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During the ten years that have passed since W. F. Libby proposed the radiocarbon method of age determination several thousand dates have been reported. Although the large majority of these measurements seem to provide accurate estimates of absolute age of events that have occurred during the past 40,000 years, a few of these measurements are clearly anomalous. One of three explanations may account for the apparent errors: (1) sample radiocarbon concentration has been wrongly measured; (2) the sample dated does not represent the event it is supposed to date; or (3) the basic assumptions of the radiocarbon age method do not apply to the particular sample.

Since the adoption of gas-counting techniques by most radiocarbon laboratories, the first source of error – namely, faulty activity measurement – has almost disappeared. Enough cross-checks between laboratories have been carried out to substantiate this conclusion; in addition, internal electronic checks and duplicate measurements of each sample keep a laboratory in day-to-day contact with the performance of its equipment.

The second source of error – misassociation of a sample and an event – is solely of human origin. It may involve mislabeling, label switching, or a similar mistake in handling. According to archeologist Frederick Johnson, "barring mistakes by collectors and laboratory workers, the very large majority of the errors are traceable to the process of selection and collection of samples. Such range all the way from faulty observation of conditions in the ground to controversy over the significance of a given stratum" (W. F. Libby, 1955).

In order to evaluate the third source of error, a sample thought to be erroneously dated must be examined in relation to the two basic assumptions of the radiocarbon method. These assumptions are: (1) the radiocarbon concentration of a particular sample when it was part of a living organism is predictable directly from that of similar material living today; and (2) the C^{14} concentration in the carbon of the sample has not been altered since formation by any process other than radioactive decay.

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The Sections of Biology and Psychology held a joint meeting on April 14, 1958, at which Lawrence E. Hinkle, Jr., The New York Hospital-Cornell University Medical Center, New York, N. Y., delivered a paper entitled "Methods of Interrogation and Indoctrination Used by the Communist State Police." This paper will not be published in the *Transactions*.

The first assumption involves such problems as the constancy of cosmic ray flux with time (W. Elsasser *et al.*, 1956), isotopic uniformity of the active carbon reservoir – that is, atmosphere-hydrosphere-biosphere (H. Craig, 1954) – and reservoir dilution by radiocarbon-free combustion carbon dioxide (H. E. Suess, 1955). If any of these were to invalidate the first assumption to some degree, the error would probably be a systematic one; therefore it is not the sort of error being considered here.

It is the second assumption on which attention is to be focused in this paper. There are three main theoretical mechanisms by which a sample's isotopic composition can be altered: (1) isotopic exchange with environmental carbon; (2) isotopic fractionation as a result of decay; and (3) contamination with foreign carbon of different carbon-14 concentration. In the first case, the air or the water solutes surrounding a carbonaceous object might exchange carbon atoms with the object, thereby enriching or depleting radiocarbon content. In the second case, gaseous or soluble products from decaying organic material might carry off a disproportionate amount of one carbon isotope, thereby enriching the residue in the other isotopes. In the final case, a sample may have been contaminated by macroscopic or microscopic carbonaceous materials entering its porous structure and so escaping visual detection. Most frequently such contaminating material would be higher in radiocarbon concentration, resulting in a measured age younger than the true age.

From what is known of isotopic exchange and fractionation processes, the first two sources seem of little importance in significantly altering the isotopic composition of organic samples (W. F. Libby, 1955). Such is not the case, however, for contamination. The organic materials that comprise the bulk of dated samples are often preserved by burial in an environment charged with foreign carbonaceous material – either in the form of solid humus or rootlets or as organic solutes borne by ground water. Although the removal of rootlets is standard practice in a radiocarbon laboratory, there is a limit to the potential completeness of such removal. Beyond that point, the removal of any contaminant must be strictly chemical.

So far, the discussion has been completely theoretical. The next question is: How can contamination be recognized? Among the situations suggesting sample contamination are the following: (1) different sample types from within the same stratigraphic unit yield widely divergent ages; (2) samples in known stratigraphic sequence show inversions in age; and (3) a sample is definitely associated with an historical event, yet its age disagrees with the date of that event as known from written record.

An interesting illustration of obvious contamination is a couple of charcoal-bone pairs from an archaeological sequence investigated by W. D. Strong and associates of Columbia University. The charcoal and bone of each pair were found together and should give the same age. The resulting carbon-14 dates appear in TABLE 1.

TABLE 1

C^{14} AGES OF CONTEMPORANEOUS CHARCOAL-BURNED BONE PAIRS

Sample no.	*Charcoal date*	*Bone date*	*Sample supplier*
L 385 B	4340 ±220	3310 ±140	W. D. Strong
L 385 C	4060 ±220	2900 ±470	W. D. Strong

That each bone age is lower than its corresponding charcoal age by about the same amount is probably coincidental. The problem then becomes one of determining whether bone or charcoal yields the more reliable age. The charcoal ages are to be preferred for three reasons:

(1) If the bone ages were correct, then the charcoal must have been contaminated by older material. A mechanism can be devised for such a contaminating process, but it is exceedingly more improbable than contaminating the bone with younger carbon from percolating ground water.

(2) Bone is extremely porous and therefore is likely to hold extraneous carbon.

(3) Since the carbon content of bone is relatively low, a given amount of contamination will introduce a much larger error than in the case of carbon-rich charcoal.

With regard to field evidence presented to illustrate sample contamination, the purpose of this paper is not to discuss such controversies involving radiocarbon dates and stratigraphic correlations. It can be said, however, that here is the source of most criticism of the radiocarbon method by certain Pleistocene geologists (C. B. Hunt, 1955; E. Antevs, 1957). Although such criticisms may be extreme, they emphasize the need for contamination studies such as are reported in this paper.

As to conflicts between historic and radiocarbon dates, the number is not large; if it were, the method would not be accepted as widely as it is. In fact, Libby's first work was designed to determine whether he had a dating method by comparing with historically dated events. As time goes on, historic checks continue to validate the method. One recent historic check made at the Lamont Observatory involved the dating of burned bread remains charred when Pompeii was overwhelmed by volcanic ash in 79 A.D.; the result was an age within fifty years of this date.

Returning to the question "How can contamination be recognized?", if a given sample date actually cannot be checked by either historic or other radiocarbon dates or by incontrovertible field evidence, can the question of contamination then be answered? Sometimes the answer is a positive one, and the method of answering involves chemical pretreatment. On the assumption that a sample is contaminated, the problem is either

one of removing the contaminant or of chemically dividing the sample into a contaminant-free fraction and a contaminant-bearing fraction. In both cases, identification of the sample contaminant is required in order to reveal its properties.

Contaminants cannot be identified by actually seeing them (those that are visible—such as coarse rootlets—are removed manually, and are not to be discussed here). It is necessary to infer the important contaminants based on both the sample type and its preserving environment. In this paper five sample types are considered: wood, peat, charcoal, burned bone, and shells. With all five, burial is the general mode of preservation. This latter fact immediately suggests three main contaminants: (1) calcium carbonate carried by ground water in bicarbonate form; (2) rootlets too small for visual identification and removal; and (3) soluble organic material also borne by ground water. We may consider each in turn.

Calcium carbonate is a problem only in the case of shells, which themselves are generally composed of calcium carbonate. Since the other four sample types are organic in nature, carbonate contaminants can be removed by leaching with dilute hydrochloric acid that hydrolyzes the calcium carbonate to carbon dioxide gas without appreciably attacking the organic material itself. With shells, the use of a surface acid leach is standard practice; unless contaminating carbonate has penetrated deeply, such treatment should be sufficient. A number of contemporaneous pairs of organic and carbonate samples bear out the general reliability of carbonate materials. Several are listed in TABLE 2.

The second type of contaminant—minute rootlets—presents perhaps the biggest problem from a qualitative standpoint. Because of their

TABLE 2

C^{14} AGES OF CONTEMPORANEOUS PAIRS OF ORGANIC AND CARBONATE SAMPLES

Sample no.	*Sample type*	*Locality*	*C^{14} age*	*Sample supplier*
L 391 D	Wood	Vancouver delta sands	11,850 ± 250	J. G. Fyles
L 391 E	Shells	Vancouver delta sands	12,000 ± 250	J. G. Fyles
L 376 D	Organic	Great Salt Lake core	26,300 ±1100	A. J. Eardley
L 376 D	Carbonate	Great Salt Lake core	25,300 ±1000	A. J. Eardley
K 101*	Wood	Glacial deposits, Denmark	10,890 ± 240	(J. Iversen, 1953)
K 113*	Lake marl	Glacial deposits, Denmark	10,930 ± 300	(J. Iversen, 1953)

*Dated by Copenhagen Laboratory (E. C. Anderson *et al*, 1953).

chemical similarity to wood and peat, small rootlets are virtually impossible to separate chemically. The fractionation treatment described later, however, is a possible method by which to detect such rootlets. Fortunately, many samples are buried deeper than the root zone and so are unaffected. Even samples available to rootlet penetration generally should be reliable because the percentage of rootlet material that escapes detection and removal is probably quite small.

Last, and probably most important of all, is the type of contamination with soluble organic materials. These substances, broadly referred to as humic acid, are apparently a family of complex organic compounds derived from decaying vegetation (W. G. C. Forsyth, 1947). Although there are conflicting opinions regarding the details of both the origin and the molecular structure of humic acid, there is agreement as to its properties. It is slightly water-soluble, forming mildly acidic solutions (*p*H around 4). With alkali metals (for example, sodium and potassium), it forms soluble salts; with an alkaline-earth metal such as calcium, insoluble salts. In solutions of low *p*H (that is, moderate to strongly acid), humic acid forms a gelatinous dark precipitate that becomes a black shiny solid when dried (E. J. Russell, 1937). Particularly important to the problem of contamination are its mobility in the soil and its relatively great resistance to natural decomposition. These facts about humic acid suggest both its potential contaminating power and chemical methods for its removal from a sample.

At the Lamont Radiocarbon Laboratory, pretreatment of all organic samples for removal of humic acid has been the general rule for the last year. The procedure is outlined in FIGURE 1. Percentages shown are by weight. The base treatment for solubilizing humic acid as sodium humate lasts for one half hour, while the acid treatment requires only fifteen minutes. The acid treatment serves two purposes: it neutralizes residual sodium hydroxide that could potentially pick up atmospheric carbon dioxide and it releases carbon dioxide from any carbonate material in the sample.

In every sample treated thus far, a detectable amount of humic acid has been recovered. This is due to its gelatinous, fluffy nature when precipitated in acid solution. It does not follow that a sample is contaminated simply because humic acid is obtained from it. As shown below, the humic acid recovered is generally from decay of the sample itself and so has the same carbon-14 concentration.

Although the quantity of dry humic acid recovered is usually insufficient to be dated, a number of samples have yielded amounts large enough for dating. Nine such humic dates, together with corresponding dates of the treated samples, are listed in TABLE 3. Of these nine pairs only two (L 400-A and L 400-C) show evidence of significant humic contamination. Both were contained within Alaskan permafrost layers and were released by blasting (J. C. F. Tedrow and L. A. Douglas,

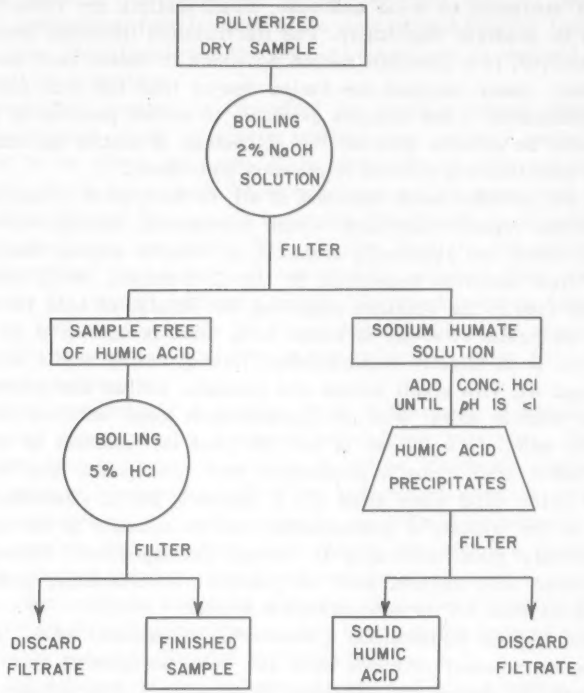

FIGURE 1. Treatment for removal and recovery of humic acid.

TABLE 3

DATES OF HUMIC ACIDS AND CORRESPONDING TREATED SAMPLES

Sample no.	*Sample type*	*Treated sample age*	*Humic acid age*	*Sample supplier*
L 400 A	permafrost soil	2900 ± 130	2000 ± 150	J. C. F. Tedrow
L 391 B	peat	4650 ± 150	4700 ± 150	J. Terasmae
L 400 C	permafrost grass-soil	8150 ± 150	5300 ± 100	J. C. F. Tedrow
L 368	peat	7350 ± 650	8350 ± 200	J. R. Williams
L 400 B	permafrost soil	10,600 ± 260	10,900 ± 280	J. C. F. Tedrow
L 163 A	lignitized wood	39,000 ±2600	39,000 ±2000	T. Karlstrom
L 414 B	wood	>32,000	> 36,000	James Thorp
L 414 A	buried soil	>34,000	> 41,000	James Thorp
L 397 E	wood	(>34,000)*	> 44,000	R. P. Goldthwait

*Dated by H. E. Suess, 1954, Sample W-96.

1958). It is interesting to note that the oldest of the permafrost soils is apparently free of humic contamination. The same is true of samples 414-A and B, a buried soil with included wood (E. Gamble, 1958). The great ages of these two samples, as well as of samples L 163-A and L 397-E, are indicative of what was known even before pretreatment began – namely, that contamination during burial is not the rule, for numerous old untreated samples have been found beyond the sensitivity of the Lamont radiocarbon counters.

Zeuner (1955) has suggested that humic acids might be removed along with the volatiles driven off in the destructive distillation of wood samples; the charcoal left behind would supposedly be contamination-free. Such a suggestion for humic acid removal seems based on the intuitive belief that humic acid, being soluble in water and hence mobile, is therefore relatively volatile. This line of reasoning is not necessarily valid. In fact, in the Lamont method of burning samples the material is first destructively distilled to form charcoal; it has been found that, of all materials so burned, humic acid gives off the smallest quantity of volatiles. There is no evidence as yet to support Zeuner's suggestion.

In addition to removing humic acid by solubilizing it with sodium hydroxide solution, it is possible to isolate chemical fractions of organic materials and then to date them individually. The fraction chemically most similar to humic acid should include this contaminant; an age difference would thus reflect the presence of contamination.

The most practical materials to fractionate are wood and peat. This is true for two reasons: (1) the chemical nature of these materials has received a great deal of investigation (S. A. Waksman and K. R. Stevens, 1928; J. Risi *et al.*, 1950; L. E. Wise and E. C. Jahn, 1952); and (2) several hundred grams of material are needed, a requirement met most frequently by wood and peat. At this point it is well to mention that whether a sample receives any type of pretreatment depends on the weight of sample available. Small samples – on the order of several grams of dry organic material – must often go without treatment in order to ensure that enough carbon is available for a radiocarbon measurement.

In selecting samples to which fractionation techniques were to be applied, the Lamont Laboratory was aided by the Geological Survey of Canada and the head of its Pleistocene Division, V. K. Prest. It was felt that the best possible study would involve a contemporaneous pair of wood and peat from an old glacial site. Accordingly, a Canadian site (J. G. Fyles, 1956) was chosen as being sufficiently old to fall in the far range of the Lamont radiocarbon counters – namely, from 25,000 to about 45,000 years ago. From this site large quantities of contemporaneous peat and wood were supplied by the Canadian Survey.

The selection of old samples was based on the fact that the error caused by a given percentage of modern contaminant increases rapidly with greater sample age. For example, a sample older than 100,000 years

would have an apparent age of 37,000 years if contaminated with only 1 per cent of modern carbon. Hence old samples, being so sensitive to even small amounts of modern contamination, are ideal for studying the contamination problem in general.

FIGURE 2 is a graph showing the degree to which the true age of a sample would be altered with various percentages of both contemporary and dead contamination (W. S. Broecker and J. L. Kulp, 1956). From this graph it becomes obvious that the closer the ages of a sample and its contaminant, the less significant will be the error in the radiocarbon age.

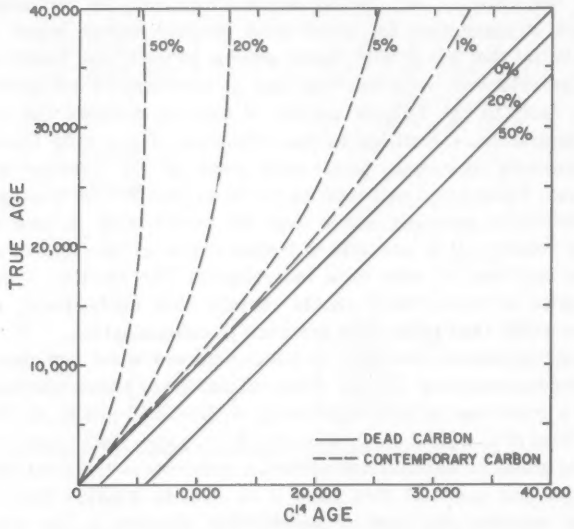

FIGURE 2. Effect of recent contamination of a sample with various percentages of contemporary or dead carbon. Reproduced by permission of Broecker and Kulp, 1956.

With old wood and peat samples available, the next step was to select the most practical chemical fractions for isolation. For quantitative reasons, these seemed to be cellulose and lignin. FIGURE 3 outlines the chemical procedure applied to each sample in obtaining the two fractions. After being ground in a Wiley mill to about 40-mesh size, each sample was divided into 2 roughly equal portions. Both were extracted with boiling distilled water (40 ml. per gram of sample) for 3 hours. This was found to remove less than 10 per cent of the sample weight, comprising certain sugars, inorganic salts, and polysaccharidic substances. Removal of constituents soluble in ether, ethanol, and benzene was not considered necessary; in fact, since these are carbon-bearing

solvents, it was considered desirable to avoid contacting them with the sample. In the future, once it is established that the organic solvents are of petroleum or coal origin and thus free of radiocarbon, such solvent extractions may provide additional datable fractions for very old samples.

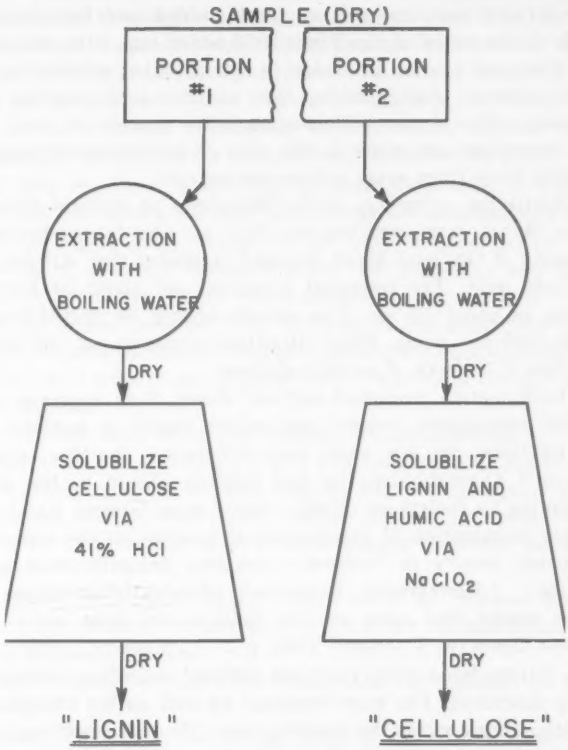

FIGURE 3. Treatment for isolating cellulose and lignin fractions of wood and peat.

After each of the two sample portions has been water-extracted, one receives a treatment for cellulose removal, the other for lignin removal. The removal technique involves chemically solubilizing either the cellulose or lignin and then separating the remaining solid sample by filtration. What remains after lignin removal is called "cellulose," although it contains other organic substances as well. So-called "lignin" is defined similarly. The solubilized portions, the true lignin and cellulose, have not been recovered thus far simply because it is inconvenient to do so.

In solubilizing lignin and cellulose, standard techniques developed by wood chemists have been followed (L. E. Wise and E. C. Jahn, 1952). Lignin solubilization is carried out in a dilute, slightly acidified, sodium chlorite solution at about 75° C. Based on 100 gm. of sample, the quantities involved are as follows: 3000 ml. of distilled water, 40 gm. of 76 per cent technical sodium chlorite added each hour for 4 hours, and 55 ml. of one normal hydrochloric acid added just after each chlorite addition. Frequent stirring is desirable. When sodium chlorite is added, frothing is extreme; consequently, slow addition with constant stirring is necessary. After 4 hours of treatment, the sample is much lighter in color, becoming pure white in the case of undeteriorated wood. Filtration using glass filter paper is then carried out.

For solubilizing cellulose, E. S. Baarghorn of Harvard University, Cambridge, Mass., suggested the use of 41 per cent fuming hydrochloric acid in place of the more harsh standard treatment that utilizes 72 per cent sulfuric acid. The treatment continues for about 24 hours at a temperature of about 10° C. The sample should be stirred frequently during the first few hours. Final filtration separates out the cellulose, which is then in the form of soluble glucose.

In the fractionation procedure outlined above, it is apparent that any humic acid contaminant present can appear wholly or partially in one of three fractions: the hot water extract solution, the "cellulose," or the "lignin." Theoretically, the last fraction should be the dominant one; according to Baarghorn (1948), whose main interest has been the microscopic examination of paleobotanical specimens, the sodium chlorite treatment results in "virtually complete delignification and 'dehumification.' " Furthermore, humic acid chemically resembles lignin to such a degree that many workers believe that most humic acid is derived from lignin (W. S. Gillam, 1940; W. G. C. Forsyth, 1947).

TABLE 4 lists dates of the fractions isolated according to the methods previously described. The wood fractions as well as the untreated wood have identical ages within the counting error. The peat fractions, however, differ slightly but significantly from each other and from the wood. These

TABLE 4

C^{14} DATES: VANCOUVER ISLAND GLACIAL SITE

Sample type and fraction	*Counts per minute above background*	*Age in years*	*Error*
Untreated wood	—	25,600	±1800
Wood lignin	2.43 ±0.20	25,850	±500
Wood cellulose	2.38 ±0.08	25,900	±300
Peat lignin	2.71 ±0.11	25,050	±300
Peat cellulose	2.99 ±0.10	23,450	±300

differences might be explained in two ways. First, contrary to theoretical expectations, contamination may have concentrated more heavily in the cellulose fraction. Second, macroscopic modern contamination, such as undetected rootlets, may have been significant; since modern material is higher in cellulose content than old peat, the cellulose fraction would be contaminated by a higher percentage. Despite these small differences, however, a date of approximately 26,000 years is indicated for the sample pair. Unfortunately, such an age requires that a large number of geologic events be compressed into a time interval which, according to J. G. Fyles of the Canadian Geological Survey, seems entirely too short. Other samples in the area are being dated to resolve this point.

After this report of preliminary contamination work at the Lamont Radiocarbon Laboratory, the need for still further work becomes apparent. Some of the experiments planned for the future include: (1) the isolation and dating of chemical fractions other than cellulose and lignin; (2) artificial contamination of old dated samples to determine the effectiveness of the above treatments for removal of the contaminant; and (3) exposure of powdered wood to highly radioactive carbon dioxide or carbonate solution in order to place lower limits on rates of isotopic exchange between phases.

At the present time, these four conclusions are indicated:

(1) Appreciable contamination (several per cent) of buried samples is the exception and not the rule. Hence, from the standpoint of sample contamination, radiocarbon ages younger than 20,000 years are more likely to be correct than in error even though there has been no pretreatment.

(2) Samples with ages beyond 25,000 years are subject to question until adequate evidence is presented to show that contamination is either absent or has been removed.

(3) Ages in error because of contamination will probably be less than the true ages.

(4) Efforts to extend the dating range of radiocarbon counters must be accompanied by the development of techniques for detection and removal of contamination.

References

ANDERSON, E. C., H. LEVI & H. TAUBER. 1953. Copenhagen natural radiocarbon measurements. I. Science. **118**: 6-9.

ANTEVS, E. 1957. Geological tests of the varve and radiocarbon chronologies. J. Geol. **65**: 129-148.

BAARGHORN, E. S. 1948. Sodium chlorite as an aid in paleobotanical and anatomical study of plant tissues. Science. **107**: 480-481.

BROECKER, W. S. & J. L. KULP. 1956. The radiocarbon method of age determination. Am. Antiquity. **22**: 1-11.

CRAIG, H. 1954. Carbon 13 in plants and the relationships between carbon 13 and carbon 14 variations in nature. J. Geol. **62**: 115-149.

ELSASSER, W., E. P. NEY & J. R. WINCKLER. 1956. Cosmic ray intensity and geomagnetism. Nature. 178: 1226-1227.

FORSYTH, W. G. C. 1947. The characterization of the humic complexes of soil organic matter. J. Agr. Sci. 37: 132-138.

FYLES, J. G. 1956. Surficial geology of the Horne Lake and Parksville map-areas, Vancouver Island, British Columbia. Ph.D. Thesis. The Ohio State University.

GAMBLE, E. 1958. Descriptions and interpretations of some Pleistocene sections in Wayne County, Indiana. Earlham College Sci. Bull. No. 3. In press.

GILLAM, W. S. 1940. The chemical nature of humic acid. Soil Science. 49: 430-453.

HUNT, C. B. 1955. Radiocarbon dating in the light of stratigraphy and weathering. Sci. Monthly. 81: 240-247.

IVERSEN, J. 1953. Radiocarbon dating of the Alleröd period. Science. 118: 9-11.

LIBBY, W. F. 1955. Radiocarbon Dating. 2nd ed. Univ. Chicago Press. Chicago, Ill.

RISI, J., C. E. BRUNETTE, D. SPRENCE & H. GIRARD. 1950. A chemical study of the peats of Quebec. Bull. Quebec Dept. Mines.

RUSSELL, E. J. 1937. Soil Conditions and Plant Growth. 7th ed. Longmans Green & Co. New York, N. Y. & London, England.

SUESS, H. E. 1954. U. S. Geological Survey Radiocarbon Dates. I. Science. 120: 467-473.

SUESS, H. E. 1955. Radiocarbon concentration in modern wood. Science. 122: 415-417.

TEDROW, J. C. F. & L. A. DOUGLAS. 1958. Carbon-14 dating of some Arctic soils. Mimeographed note. N. J. Agr. Expt. Station.

WAKSMAN, S. A. & K. R. STEVENS. 1928. Contributions to the chemical composition of peat. I and II. Soil Science. 26: 113-137; 239-251.

WISE, L. E. & E. C. JAHN. 1952. Wood Chemistry. ACS Monograph. Reinhold. New York, N. Y.

ZEUNER, F. E. 1955. Radiocarbon Dates. Eleventh Annual Report of the Institute of Archaeology. : 43-50. University of London. London, England.

THE NEW YORK ACADEMY OF SCIENCES

SECTION OF MATHEMATICS AND ENGINEERING

SOME DEVELOPMENTS IN RHEOELECTRIC SIMULATION APPLICATIONS TO HEAT-POWER-PROCESS SYSTEMS*

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Heat-power-process energy-flow systems are often of such complex character that orthodox analytical methods of a mathematical, graphical, and numerical nature prove inadequate. In general, the solution of energy-flow processes, particularly under off-design conditions, is fraught with many complications of varying component behavior. Under these circumstances, recourse to analysis and performance prediction by way of the resistance concept of energy flow offers distinct advantages. Furthermore, in terms of the concept that expresses flow as a result of a motivating force acting against resistance, processes may be simulated analytically by electric flow with its attendant circuitry, that is, through electric analogy. It is, of course, understood that in such a procedure many simplifying assumptions are introduced for expediency, and the results obtained by such analogue-computer technique are definitely to be associated with the assumed basic data.

In the field of resistance-concept analysis for energy-flow processes, especially by way of rheoelectric analogy applications, many studies have been carried on in heat-conduction problems. I refer to a variety of steady-state developments in this realm, using both geometric-sheet analogue methods[1-4] and network[5] methods. Further extension of the concept has covered the heat-exchanger problem,[6] combined heat and vapor flow,[7] and the energy-transport principle as applied to a refrigerating-plant complex.[8,9]

In this treatment, two further and separate types of problems will be considered under steady-state conditions. The first deals with a multi-branched fluid-flow pipe network with discharge flows at a number of separate locations; the second, with a heat and power complex involving a steam turbogenerator unit combined with a vapor-condensing heat exchanger to supply heated fluid to a distribution system such as encompassed in the first problem. Both of these analyses are related insofar as they involve nonlinear resistance relationships; in addition,

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each of these involves separate concepts of motivating flow force and attendant definitions of resistance.

Analysis of a Process-Fluid-Flow Distribution Network

For a process-fluid-flow network of numerous interconnected branches of different sizes and lengths, the determination of the flow distribution by calculation is difficult because of the pressure-drop versus flow-rate characteristics of each individual branch of the network. Not only does the pressure drop vary with the effective pipe and surface conditions, for a given fluid but, as well recognized, very definitely with the flow rate. Herein the concept of "flow resistance," representing opposition to the flow of fluid, is a very useful tool.

The pressure drop in any pipeline branch may be considered as proportional to the product of the flow rate and the pipeline resistance; for turbulent flow, the resistance itself may be considered a function of the flow rate. Thus, the flow characteristics of a network may be simulated through rheoelectric analogy, with the electric resistance in each branch simultaneously adjusted to some desired function of the electric current. Voltage and current determinations are representative of the corresponding fluid pressure and flow rates under steady-state conditions.

FIGURE 1 shows a steady-state fluid-flow network of many branches and different sizes, as treated in a recent paper.[10] Here hot water is supplied from a central source under specified initial supply pressure, with simultaneous distribution desired for requisite discharge rates to a number of remotely located process operations.

Fluid-flow characteristics. The pressure drop ΔP for turbulent pipe flow depends on a number of factors, but foremost here for a given length is the flow-rate Q, varying exponentially, and a friction factor f that depends on the flow rate as well as on the size and surface characteristics of the pipe. Use is often made of a modified form of flow equation for practical use in connection with a pipe of fixed dimensions transporting a given fluid:

$$\Delta P = C(Q^x) \tag{1}$$

where C is a specialized constant and x equals the exponent, variously taken as a value between 1.80 and 1.90, often as 1.85 for water.

For a given composite pipeline consisting of straight pipe lengths, together with fittings such as couplings, unions, elbows, and tees, plus necessary additions of control valves, there are, consequently, other supplemental causes of pressure drop that are thus in addition to the straight pipe flow. Various approaches are used in evaluating the valve and fitting losses; perhaps the most practical one in use considers these losses as equivalent to those occasioned by a given length of straight pipe, expressed supplementally as L_t. Hence the equivalent total pipe

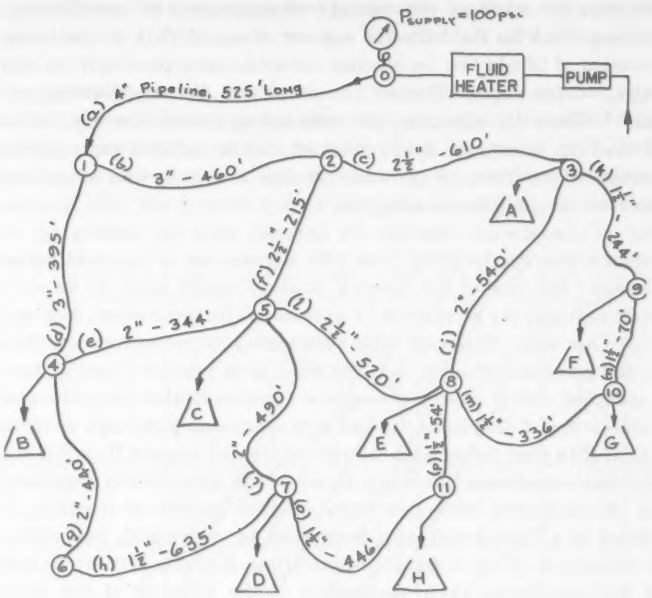

FIGURE 1

length L_e may be set up, comprised of pipe length L_p and fitting "length" L_f:

$$L_e = L_p + L_f \tag{2}$$

Basing the pressure drop for a given pipeline on a standard effective length L_e such as 100 feet, the variation of pressure drop with flow rate will be proportional for other lengths thus:

$$\Delta P = (L_e/100)\,(\Delta P_{100}) \tag{3}$$

Finally, it should be noted that, for a given pipeline exposed to an overall pressure difference ΔP across its extremes, the flow rate Q is the result. Accordingly, EQUATION 4 expresses the relationship:

$$Q = (\Delta P/C)^{1/x} \tag{4}$$

Resistance concept of fluid flow. In terms of EQUATION 1, $\Delta P = CQ^x$ for a pipe of given dimensions, the pressure drop ΔP as a motivating force may be considered to overcome a fluid-flow resistance R in transporting the fluid at a given rate Q through the flow channel. The equation may be written:

$$\Delta P = QR \tag{5}$$

and

$$R = \Delta P/Q = CQ^x/Q = CQ^{x-1} \tag{6}$$

Use may be made of the concept of resistance in establishing the motivating force for fluid flow in a given channel. This is similar to the application of Ohm's law for electric circuits, correspondingly for thermal circuits,[1-6] for vapor-diffusion circuits,[7] and for energy-transport circuits.[8,9] There is, moreover, an outstanding circumstance to be noted here: the flow resistance is not constant, but is *variable* and specifically a function of the flow rate Q. In fact, it may or may not be a linear function of the flow rate, regular or irregular.

Use of rheoelectric analogy. In general, with the setting up of the resistance concept for fluid flow with assumption of constant values of resistance, the use of the electric analogy would seem to be obvious: electric voltage, the equivalent of pressure; electric current, the equivalent of flow rate. However, with dependency of resistance on the flow rate, the problem becomes more complex. It is resolved here in terms of an accepted ΔP-Q curve, through a servocontrolled variable electric resistance that follows the desired R-Q relationship through an appropriate cam. R is thus varied with the rate of electric current flow. Utilization of the cam introduces flexibility to cover the specifically required variation of resistance with flow rate, whether regular or irregular, under command of a current-detecting instrument in series with the resistance. The utilization of the resistance-controlling elements will be understood best by considering their application to the solution of the hydraulic network represented by FIGURE 1.

Illustrative example. The hydraulic circuit shown in FIGURE 1 is supplied with hot water at 180° F from a pump-and-heater installation, with a pressure at Point 0 of 100 psi. The flow distribution system consists of 16 different interconnected branches (from *a* to *p*) of different lengths, plus accessory fittings and pipe sizes (nominal), as indicated on the diagram. At 8 different locations – Points 3, 4, 5, 7, 8, 9, 10, and 11, required discharge rates to Process Vats A, B, C, D, E, F, G, and H are to be maintained simultaneously at a required *minimum* pressure of 25 psi. The required discharge rates are listed below:

Point	3	4	5	7	8	9	10	11
Vat	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*
Q, lb./min.	250	625	800	500	500	300	250	175

Representative ΔP_{100}-Q relationships for 100 feet of effective length, conforming to the general EQUATION 1, are shown in a logarithmic plot in FIGURE 2. These are for pipes in good condition and of different sizes. These relationships are assumed, for the purposes of the problem, to represent fairly the friction circumstances of the process-network pipes. In the same figure, equivalent resistance curves R-Q are shown. (In the absence of a standard designation for the unit of flow resistance,

for convenience here, as suggested by electric practice, the term flohm is used, where 1 flohm = 1 psi per 100 feet per unit flow rate of 1 lb./min.)

The hydraulic circuit of FIGURE 1 is simulated in electric analogy with each of the 16 branches represented by an automatic-control current-measuring instrument, cammed to self-adjust the value of a variable resistance in series with it and representing the pipeline resistance in accordance with the desired R-Q relationship. In setting up the electric

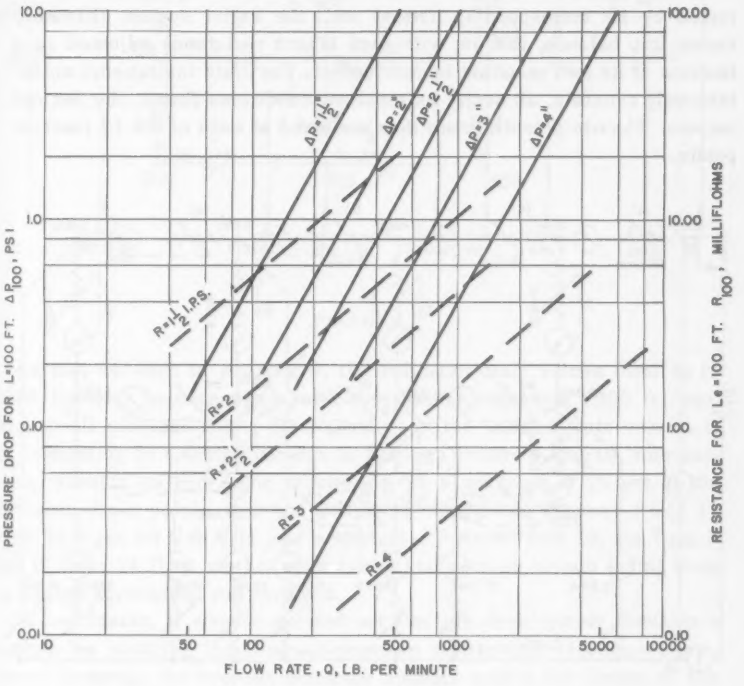

FIGURE 2

analogy for this computer circuit, the following conversion equivalents are employed, with the hydraulic circuit of FIGURE 1 reproduced as an electric network:

Voltage: 1.00 volt = 10 psi; current: 1.00 ma = 50 lb./min.;
Resistance: 1.00 ohm = 0.000200 flohms = 200 microflohms.

The equivalent computer circuit is shown in FIGURE 3 and includes the 8 drain points. These required discharges, representing in effect and

total the output flow of the entire system, are served by adjustable constant-current instruments. Each instrument is set for the requisite amount and serves to control a variable resistance to the low side, or "ground," in meeting its constant current requirements. Across the over-all network circuit, that is, between Points 0 and 12, a direct-current voltage of 10.0 volts is imposed. As a result, currents tend to flow through all the 16 different branches and out of the 8 drain outlets to meet the imposed current requirements. Progressively throughout the entire network, each "pipe-branch" resistance is continually self-adjusted by its corresponding current until the entire system ultimately comes into balance, that is, with each branch resistance adjusted as a function of its own resultant branch current. The drain instruments simultaneously maintain, as nearly as resultant conditions permit, the desired outputs. Electric potentials are then measured at each of the 12 junction points.

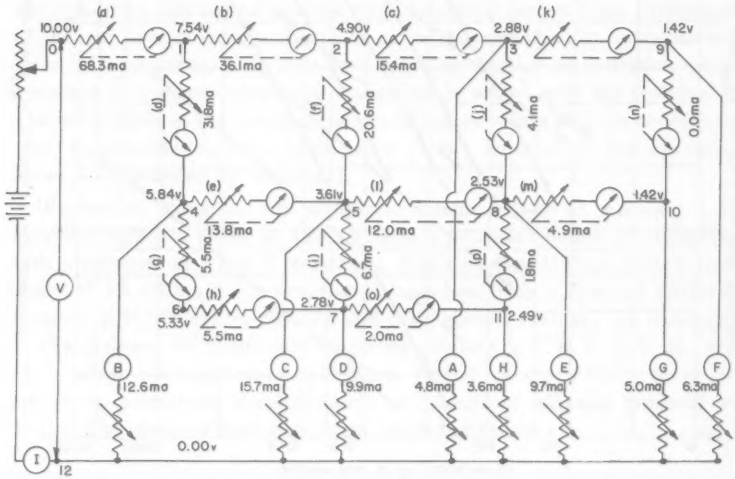

FIGURE 3

In carrying out this study, in addition to determining the voltage at each of the junction points, the different branch currents were read via the indicating current meters. The true current was obtained via the meter indication and its instrument-shunt calibration. Equivalent hydraulic data, to cover the resultant performance prediction of the network under the imposed conditions, followed from the electric data and are shown, with pertinent branch-line information, in FIGURE 4. The branch-line information covers the effective length L_e, inclusive of fitting-and-

accessory value L_f, and may be compared with the L_p information of the hydraulic circuit, FIGURE 1.

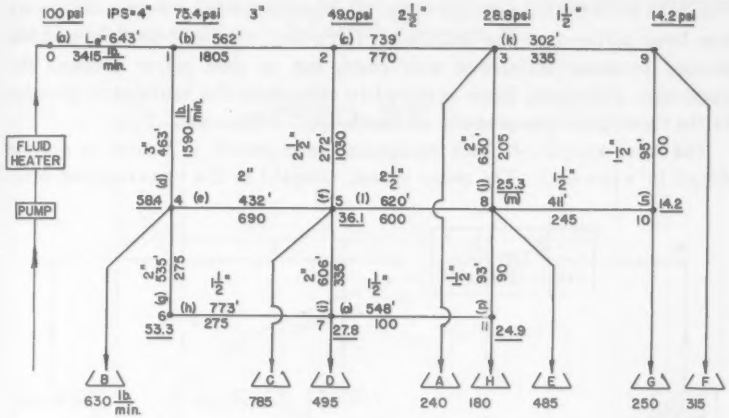

FIGURE 4

As may be seen in FIGURE 4, the indicated drain values total up to 3380 lb./min. in comparison with the setting values of 3400 lb./min. with small plus and minus discrepancies in the various drain values. It is interesting to note that Branch *n*, between Points 9 and 10, was useless, passing no flow. The requirement of a minimum of 25 psi at the different drain points was not completely satisfied. Whereas Point 11 with 24.9 psi for Vat *H* is just acceptable. Points 9 and 10, for Vats *F* and *G*, with 14.2 psi are not acceptable, and network branch sizing must be further scrutinized and revalued.

In conclusion, it may be pointed out that the development provides a method for studying the flow-and-pressure distribution in complex nonlinear systems; its over-all accuracy appears within the limits of the employed fundamental-data accuracy; and it is readily adapted to a wide range of resistance versus current requirements.

Performance Prediction for a Process Heat-and-Power Complex

In the heat-and-power process plant herein discussed, a prime mover, such as a steam turbine, exhausts directly to a vapor-condensing fluid heater, thus serving, in effect, as a power-producing "reducing valve." In such an arrangement, the performance of the turbine as to steam consumption and corresponding steam energy exhaust is greatly influenced by the operating pressure on the heater; conversely, the heater perform-

ance is directly dependent on the associated steam-turbine performance. The prediction of over-all performance characteristics of such an integrated complex is one in which the resistance concept is again of unique value. In heat conduction and other such problems temperature difference has been utilized as the motivating force and, in the fluid-flow problem above, pressure difference was used, but in this power process the particular motivating force selected to effectuate the resistance concept is the thermodynamic property of "enthalpy" difference.

The heat-and-power plant equipment arrangement is shown in salient detail in FIGURE 5. The prime mover, coupled to the power-output elec-

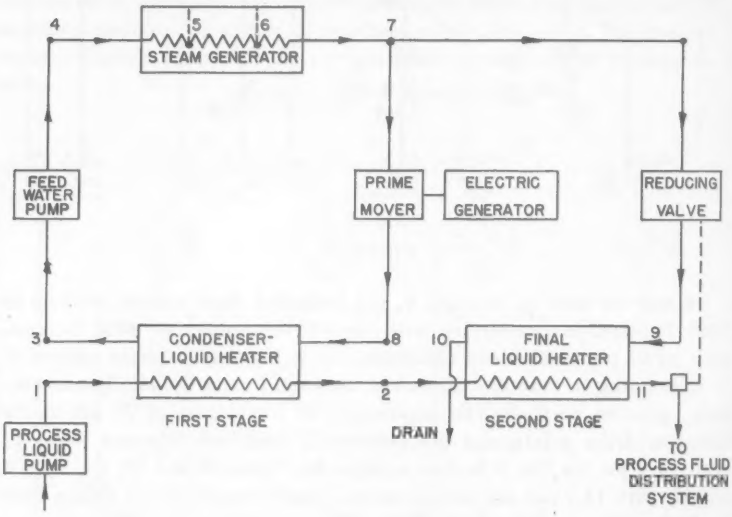

FIGURE 5

tric generator, exhausts directly to the vapor-condensing fluid heater. With fluid flow of a given rate and inlet temperature, and a heat exchanger of given characteristics, the fluid will be heated to some resultant temperature. If it is required that the fluid achieve some specified final temperature, as for the process-fluid distribution network above, a second-stage heater taking live steam through a temperature-controlled reducing valve may be incorporated into the flow circuit for this purpose. The primary interest here is in the steam circuit of the prime mover and the connected first-stage heater.

Heat exchanger relationships. If one conceives of a perfect fluid heater, the outlet fluid temperature would be that of the condensing-vapor source. Accordingly, in dealing with energy transfer in terms of enthalpy-potential

difference Δh, the *fluid enthalpy* h_f corresponding to the vapor temperature may be utilized as the source level of energy flow. FIGURE 6 incorporates the corresponding relationships from the enthalpy (total heat) viewpoint, as the counterpart of the conventional temperature relationships, and is given in extended detail elsewhere.[11]

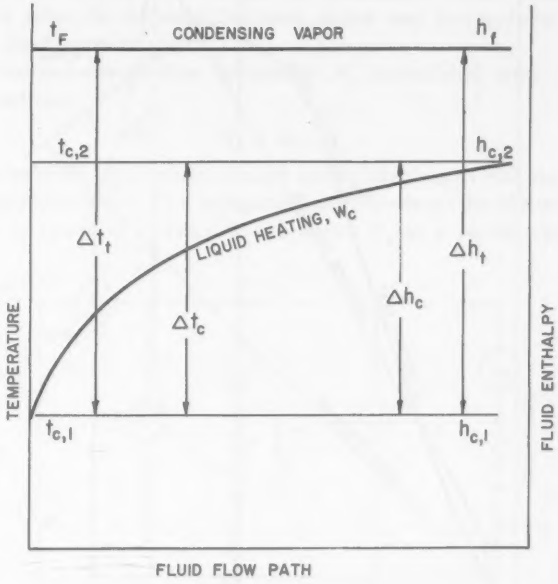

FIGURE 6

The hourly energy transfer q_F paralleling the treatment set up originally in terms of temperature[6] may be set up via resistance R_t on an enthalpy-difference base Δh_t for the heater:

$$q_F = \Delta h_t / R_t \tag{7}$$

$$R_t = R_c / (1 - e^{-R_c / R_{uA}}) \tag{8}$$

$$R_c = 1 / W_c \tag{9}$$

$$\Delta R_t = R_t - R_c \tag{10}$$

W_c is the rate of process fluid flow in lbs./hr.; $R_{uA} = 1/uA$, where uA represents the heat transfer factors of the heater (u=B.T.U./(hr.-sq. ft.-Δh_m) and A = sq. ft. transfer area).

Power cycle relationships. For a power-plant cycle, the magnitude of the back pressure has a significant effect on the efficiency of transformation of the available Δh_T into work Δh_w, and thus a corresponding

effect on the magnitude of the exhaust energy Δh_e. This is illustrated in the h-s (Mollier) diagram, FIGURE 7. With decreasing values of back

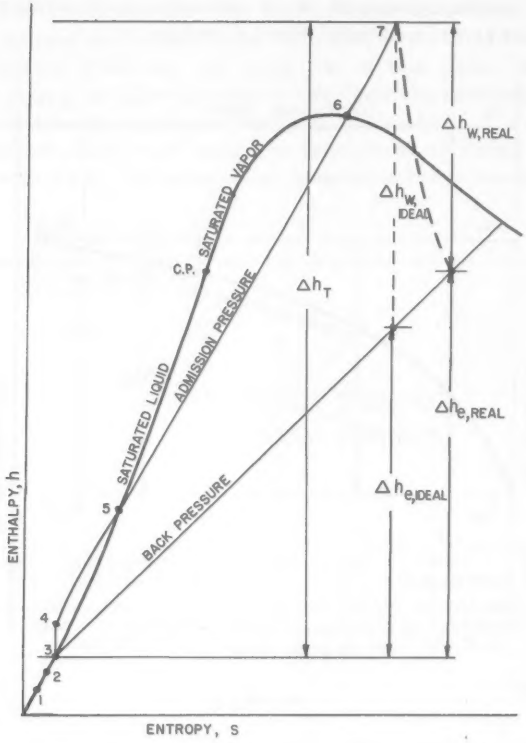

FIGURE 7

pressure, and correspondingly also of the liquid-condensate enthalpy level h_f (the bottom cycle terminus for the power process), the energy developing as work Δh_w increases. Distinction is made between ideal-cycle versus real-cycle performance (with internal losses involving efficiency η). It is also to be noted that the available work per pound of cycle fluid leads to the very significant rate-of-steam-consumption value, that is, the familiar "water rate," the hourly steam-flow rate per unit of kilowatt-hour power production. Thus

$$\text{Water rate } WR = 3415/(\eta \Delta h_{w,\,\text{ideal}}) \tag{11}$$

In previous studies on energy transport via machines[8] such as refrigeration compressors, the potential difference associated with the resistance

to energy flow was taken as temperature difference Δt. In the present analysis, inasmuch as work energy asserts itself in terms of enthalpy-potential difference Δh_T (based on h_t), it has been desirable to develop the resistance concept with the motivating force taken as this value of Δh_T. Thus, for the turbine-heater combination, such value of Δh_T is additive to the Δh_t value across the connected heater, ultimately to give the total Δh between top-level steam and bottom-level entering process fluid to the heater.

The exhaust-energy-flow resistance R_e associated with Δh_T may now be defined:

$$R_e = \Delta h_T / q_e \qquad (12)$$

q_e represents the total hourly exhaust energy discharged from the turbine.

Energy-flow circuit. The simulating electric circuit for the power-heat complex is shown in FIGURE 8, and shows R_e as a variable resistance

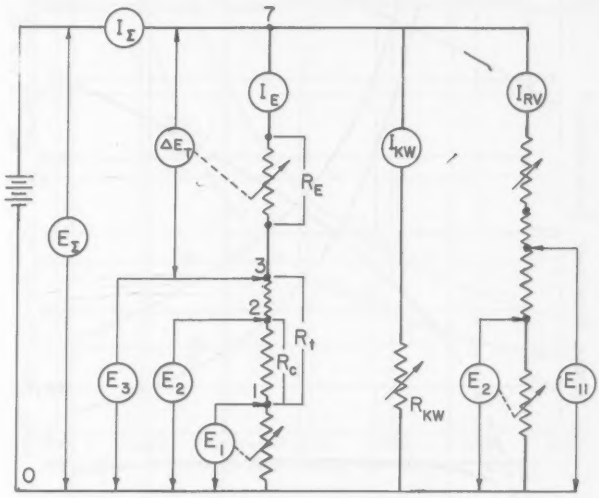

FIGURE 8

in the machine-heater section, coupled to the heat-exchanger resistance R_t defined in EQUATION 8. Electric resistance values are in scale relationship to the respective thermal resistance values. (In the absence of a standard designation for the unit of energy-flow resistance, for convenience here and as suggested by electric practice, the term enthohm is used.)

Inasmuch as R_e actually depends on the resultant value of Δh_T realized under circuit conditions, the circuit shows an automatic servocon-

trolled resistance value R_e, its characteristic depending on the particular turbine involved in the study. It must specifically be pointed out that whatever exhaust energy as such flows through the turbine must also "flow" through the heat exchanger down to the level of the incoming process fluid at Point 1 in the diagram. It is also to be noted that the circuit is truly an energy-flow circuit, just as the name indicates.

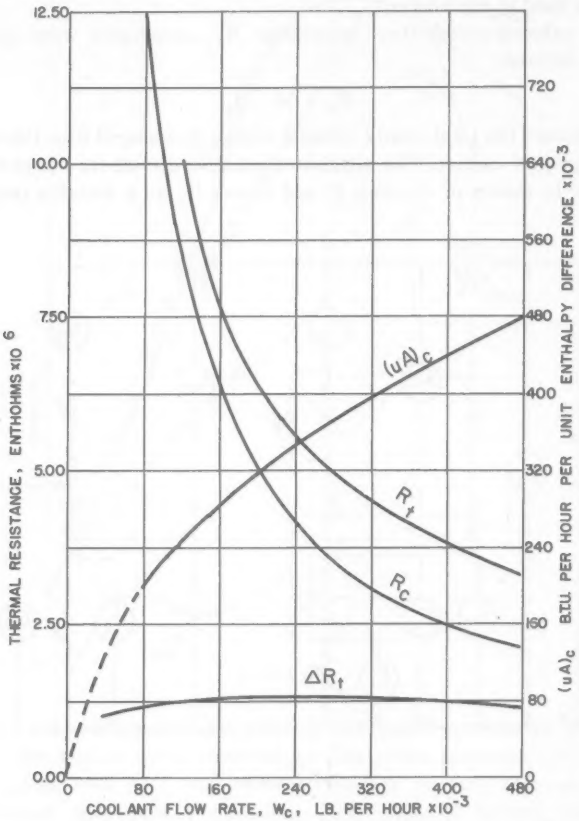

FIGURE 9

With "ground" representing 0 value of the enthalpy scale, and an appropriate DC voltage applied to represent the initial steam enthalpy h_g for Point 7, the initial fluid enthalpy $h_{c,1}$ (Point 1) may be established either by manual control of the adjusting resistance, or through servo-

control thereof via voltage E_1. Under balance conditions, E_3 represents the intermediate condensing-temperature enthalpy h_f, and E_2 the voltage indicating $h_{c,2}$ of the outlet process fluid.

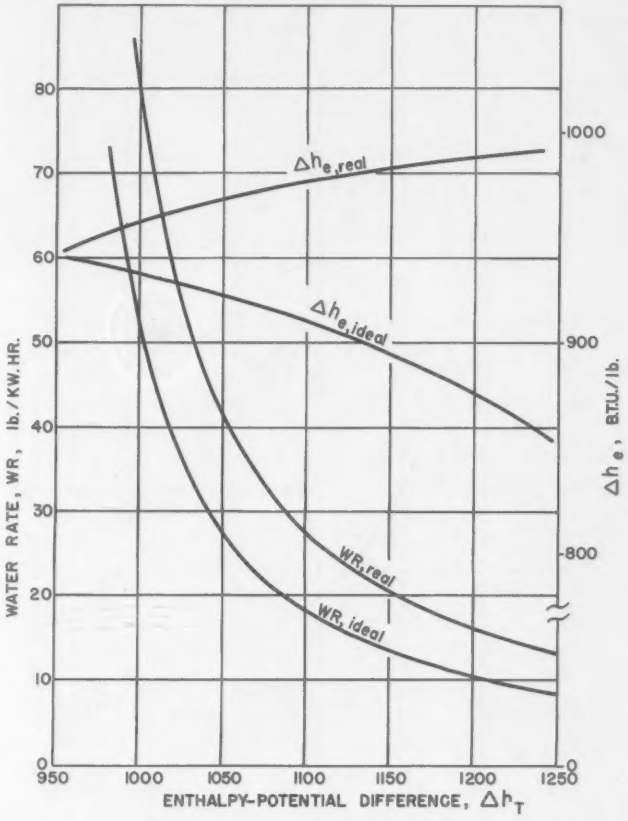

FIGURE 10

Since the reference value of kilowatt output is constant, with the only variation for the turbine set taking place in the exhaust-energy condenser-heater section of the circuit, this section is the one of particular interest. The energy flow in the power circuit is regarded as constant, and may be so set, with an automatic current regulator to maintain it so, if desired. Although it is not of primary interest here, the electric diagram also shows the additional provisions for the further heating of the process fluid in a second-stage heater to some desired value. This uses a heater

circuit similar to that of the primary heater. The required outlet potential via E_{11} serves to direct the necessary current flow through a variable resistance that simulates a pressure-reducing valve, with the second-stage inlet fluid constantly regulated to coincide with E_2 either manually or automatically.

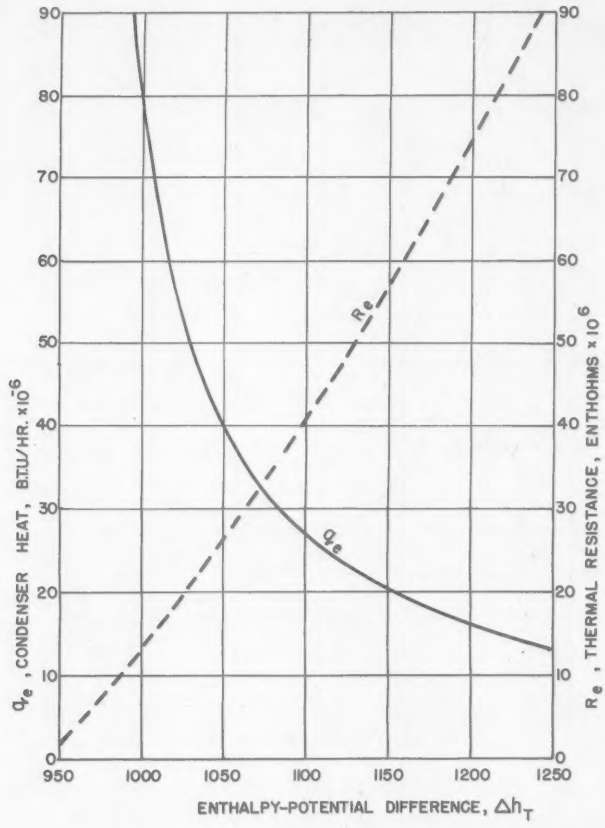

FIGURE 11

Whereas in this heat-power analogue the adjustment of the different controlling resistances to maintain values of h and of R_e as a function of Δh_T may be accomplished manually, still this is a matter of manual dexterity, greatly simplified, as noted, by the use of automatic equipment.

Illustrative example. The citation of an illustrative example employed in an earlier presentation[11] will serve to clarify the operations further.

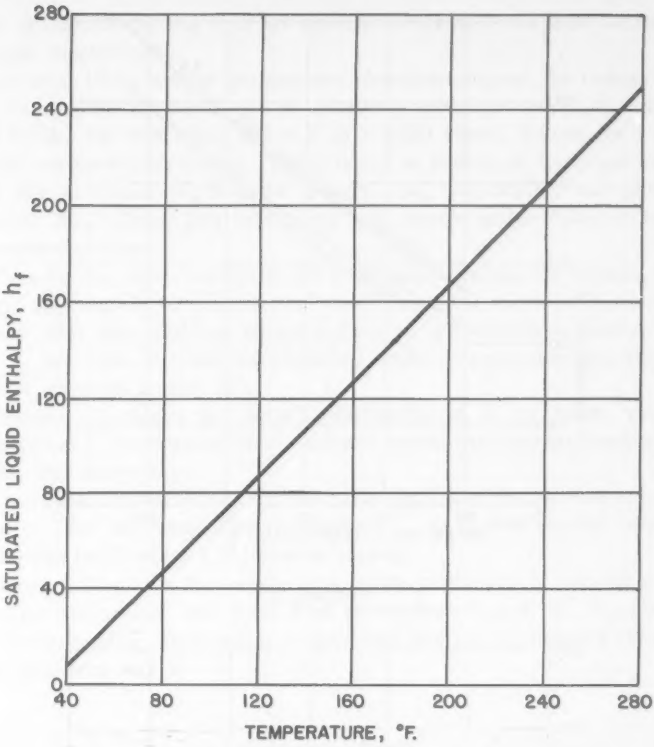

FIGURE 12

A system such as that represented by FIGURE 5 operates with steam at 250 psi and 200° F. superheat (h_g = 1319 B.T.U./lb.), and employs a base-load turbine designed to have a continuous output of 1000 kw. under the varying back-pressure conditions that might be realized. Internal turbine efficiency, η = 0.65, may be considered as constant over the range of back-pressure variation. The condenser-heater consists of 2250 sq. ft. of surface. Required: (a) plant performance predictions as to condensing temperature t_F and fluid-outlet temperature $t_{c,2}$ are desired at a process-fluid flow rate of W_c = 335,000 lb. (water) per hour and varying inlet-water temperatures $t_{c,1}$ from 40° to 130° F.; and (b) under constant inlet-water temperature of 60° F., the variations of condensing temperature t_F and of outlet-water temperature $t_{c,2}$ are required for varying water-flow rates from 200,000 to 480,000 lb. per hr.

FIGURE 9 shows the operating characteristics of the condenser heater, from which the value of R_t and the intermediate value of R_c for the flow

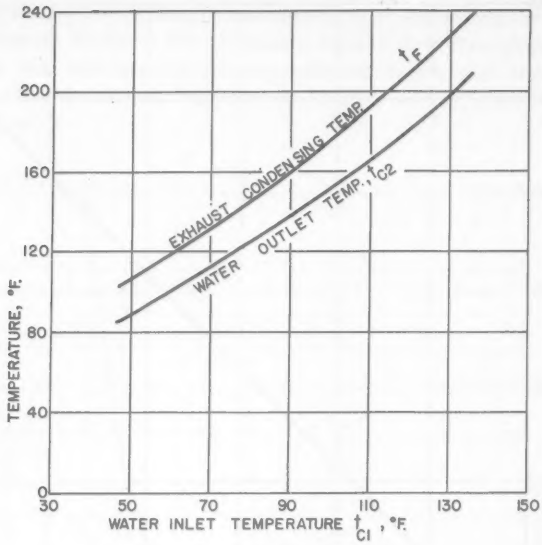

FIGURE 13

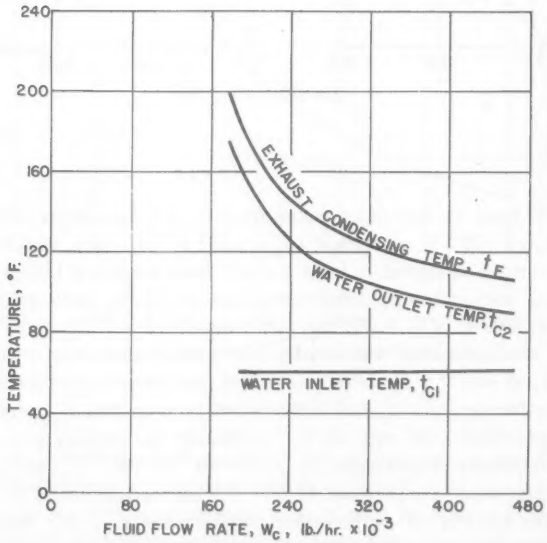

FIGURE 14

rate of 335,000 lb. per hour are determined as 4.27 and 2.98 micro-enthohms, respectively.

FIGURE 10 shows the performance characteristics of the turbine under fixed admission conditions as previously specified, that is, $h_g = 1319$ B.T.U./lb. for both ideal and real ($\eta = 0.65$) cases. Values for 1 kw. output are specified, namely "water rate," in pounds of steam per kwh., and the corresponding exhaust energy, Δh_e B.T.U./lb., and plotted against Δh_T. Thus, this represents performance under different back-pressure conditions.

FIGURE 11, then, translates the performance values for ultimate use in the analysis. It shows, for the "real" case, the variation of exhaust energy from the 1000-kw. output turbine to the condenser heater, q_e B.T.U. per hour, and the corresponding value of exhaust-energy resistance R_e, plotted against Δh_T.

FIGURE 12 shows the actual relationship of h, for water, versus temperature t, for translation of analysis results from enthalpy-potential values into temperature.

FIGURE 13 shows results for the condenser-heater outlet-water temperature $t_{c,2}$ and the condensing temperature t_F, as plotted against varying inlet-water temperature $t_{c,1}$ (required task *a*).

FIGURE 14 shows the predictions, under conditions of varying process-fluid flow rates, with fixed inlet temperature $t_{c,1}$ of 60° F., giving the corresponding outlet-water temperature and the condensing temperature (required task *b*).

Acknowledgment

Acknowledgment is made to my associates who have cooperated on various aspects of these studies, particularly to J. A. Balmford, Electrical Engineering Department, Columbia University, who jointly performed the work of the first study with me, and who generously contributed his help in connection with the second.

References

1. KAYAN, C. F. 1945. An electrical geometrical analogue for complex heat flow. Trans. Am. Soc. Mech. Engrs. 67: 713-718.
2. KAYAN, C. F. 1946. Temperature patterns and heat transfer for a wall containing a submerged metal member. Refrig. Eng. 46: 533.
3. KAYAN, C. F. 1948. Fin heat transfer by geometrical and electrical analogy. Ind. Eng. Chem. 40: 1044.
4. KAYAN, C. F. 1950. Electrical analogger studies on panels with imbedded tubes. Trans. Am. Soc. Heat Vent. Engrs. 56: 205.
5. KAYAN, C. F. 1949. Heat-transfer temperature patterns of multi-component structure by comparative methods. Trans. Am. Soc. Mech. Engrs. 71: 9-16.

6. KAYAN, C. F. 1951. Heat exchanger analysis by electrical analogy studies. Heat Transfer Discussion. London, England.
7. KAYAN, C. F. 1955. The wet-bulb process; combined heat transfer and vapor diffusion, by electrical analogy analysis. 9th Intern. Congr. Refrigeration. Paris, France.
8. KAYAN, C. F. 1953. Electrical analogger application to the heat pump process. Trans. Am. Soc. Heat. Vent. Engrs. 59: 361.
9. KAYAN, C. F. 1956. Electrical analogger analysis of a cooled structure complex. Trans. Am. Soc. Heat. and Air Conditioning Engrs. 62: 197-212.
10. KAYAN, C. F. & J. A. BALMFORD. 1957. Analysis of a process-fluid-flow network by electrical analogy. Trans. Am. Soc. Mech. Engrs. 81.
11. KAYAN, C. F. 1958. Performance prediction for a process heat-and-power complex by resistance concept. Trans. Am. Soc. Mech. Engrs. 82.

DIVISION OF MYCOLOGY

CAPSULE SYNTHESIS BY *CRYPTOCOCCUS NEOFORMANS**

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Introduction

Cryptococcal meningitis and meningoencephalitis are late clinical complications of primary pulmonary cryptococcosis, a disease that is usually benign and self-limited. In a few individuals with low host-resistance, leukemia, or other lymphomatous disease, *Cryptococcus neoformans* may disseminate from its limited focus in the lungs to other organs. For some unknown reason, *C. neoformans* possesses an unusual affinity for the central nervous system. Once the yeast has broken through the blood-brain barrier and has seeded the cerebrospinal fluid, it is distributed by the cerebral circulation to all surfaces of the brain and spinal cord. There the organism proliferates readily and forms huge capsules that render it relatively immune to the normal defense mechanisms of the host. The pathological reaction incited in the meninges by the yeast gives rise to obstructive leptomeningitis and intracranial block. As the disease progresses, pressure of the spinal fluid steadily rises, protein content increases, the cells become more numerous, and the sugar content steadily diminishes as the yeasts consume the carbohydrates and carbohydrate derivatives of the spinal fluid.

When the disease has reached the central nervous system, the prognosis becomes extremely grave. Although a patient with fulminating meningoencephalitis may expire within two weeks, the usual duration of the illness is four to six months, ending invariably in death. Chronic infections that persist for years are on record, but these also terminate fatally. A comprehensive description of the clinical and pathological features of cryptococcosis may be found in a recent monograph by M. L. Littman and L. E. Zimmerman.[1]

Despite the discovery of the polyene antibiotic, amphotericin *B* (Fungizone), and its proved value in treatment of the extraneural deep mycoses,[2-7] continued experience with it in the treatment of cryptococcal meningitis has been disappointing,[8] although initial clinical reports have been favorable.[9-11] Much of the difficulty in treatment is due to the fact that amphotericin *B* fails to reach the spinal fluid in fungicidal concentration during or following intravenous administration. In addition,

*This paper, illustrated with slides, was presented at a meeting of the Division of Mycology on April 25, 1958.

This investigation was supported in part by a grant from the Squibb Institute for Medical Research, New Brunswick, N. J.

only minute amounts of the antibiotic can be safely introduced by way of the intrathecal route. The problem of effective therapy of cryptococcal meningitis, therefore, remains urgent.

If one studies the *in vivo* and *in vitro* characteristics of *C. neoformans*, certain biological phenomena are evident. One of these, as already noted, is the predilection of *C. neoformans* for the central nervous system, particularly for the spinal fluid and meninges. Another is the considerable loss of capsular substance suffered by the organism upon transfer from its *in vivo* site to an artificial culture medium. Still another is the rapid synthesis *in vivo* of huge capsules by ordinarily weakly encapsulated laboratory strains upon intracerebral injection of experimental animals such as the white Swiss mouse. Contrary to common belief, continued maintenance of *C. neoformans* on carbohydrate-rich culture media will not restore the capsule to *in vivo* size. One has only to compare the morphological appearance of several strains of the organism to observe the considerable disparity in the size of the *in vivo* and *in vitro* capsules. In brief, it is as though *C. neoformans* has found some essential metabolites or optimal conditions in the brain and spinal fluid that furnish the stimulus for growth and the synthesis of large capsules.

In the course of an evaluation of the vitamin requirements of *C. neoformans*, the organism was cultivated in an inorganic salt medium containing ammonium sulfate as the sole source of nitrogen, with glucose, maltose, and sucrose as the sole separate sources of carbon. It was observed with India ink mounts that increasing concentrations of thiamine stimulated the synthesis of capsular substance. Investigation of this phenomenon led to a study of the vitamin, amino acid, and carbohydrate requirements of *C. neoformans*, their effects on capsule synthesis, and their relation to the *in vivo* phenomenon.

Materials and Methods

Organisms and culture media. Forty strains of *C. neoformans* isolated from human cases of cryptococcal meningitis and from pigeon excreta were employed in this study. The organisms were maintained at 37° C. on slants of yeast extract agar* and on Sabouraud's dextrose agar (Difco) enriched with biotin and thiamine. Vitamin-depleted strains were maintained at 20° C. on vitamin-free basal agar. The basal salt medium (BSM) employed for assimilation studies had the following composition: KH_2PO_4, 2 gm.; $(NH_4)_2SO_4$, 2 gm.; $MgCl_2 \cdot 6H_2O$, 0.2 gm.; $CaCl_2 \cdot 2H_2O$, 0.02 gm.; $FeCl_2 \cdot 4H_2O$, 0.04 gm.; $MnCl_2 \cdot 4H_2O$, 0.0015 gm.; $Na_2MoO_4 \cdot 2H_2O$, 0.0015 gm., all dissolved in 1 liter of $KMnO_4$-oxidized double-distilled water, after which the medium was adjusted to neutrality with 1N NaOH. The medium was then distributed in 10 ml. amounts to chemically clean, matched, photoelectric test tubes (Lumetron), covered with aluminum

*Containing per liter of medium: powdered yeast extract, 3 gm.; peptone, 5 gm.; glucose, 10 gm.; agar, 20 gm.; and pH 5 to 6.

caps, and autoclaved at 118° C. for 12 min. After seeding, tubes were incubated undisturbed at 37° C.

Vitamin-free washed agar was prepared by leaching 20 gm. of dehydrated agar (Difco) in 1 liter of double-distilled water at 5° C. for eight 5-day periods. The final, vitamin-free, washed agar was suspended in 4 per cent concentration in double-distilled water, melted, and autoclaved at 121° C. for 15 min. Slants of vitamin-free basal agar were prepared by combining washed agar and 1 per cent glucose with basal synthetic medium.

Carry-over of vitamins and metabolites in subcultures was reduced by preliminary treatment of each culture. Strains used for vitamin studies were subcultured to vitamin-free basal agar slants until the growth of the culture had become sparse. The vitamin-depleted cells were then suspended in vitamin-free BSM. One drop (0.07 ml.) of this suspension, with a light transmission of 94 per cent at 650 mμ. (Lumetron) served as the inoculum for 10 ml. of medium.

Cultures employed for carbon assimilation studies were first depleted of their carbon reserves. This was accomplished by transferring one loopful of growth from a yeast extract agar slant to carbohydrate-free liquid BSM and incubating for 72 hours at 37° C. One drop of this suspension served as the inoculum for each tube. The effectiveness of depletion techniques was confirmed by the absence of growth in seeded blanks.

Vitamin solutions were sterilized by filtering them through sterile, chemically clean, ultrafine, sintered-glass bacterial filters, after which they were added aseptically to the basal synthetic medium. All experiments were conducted with glassware cleaned in sulfuric acid dichromate and rinsed in double-distilled water. Mineral salts, vitamins, amino acids, and carbohydrates used in nutrition studies were of reagent grade and were obtained from a variety of commercial sources.*

Measurement of growth. Growth responses of *C. neoformans* to vitamins and other additives in BSM were followed photometrically at 650 mμ. after 7, 14, and 30 days at 37° C. incubation, and half-maximal values of the vitamins were computed from growth curves. Ocular micrometer measurements were made of the capsule and cell diameters with fungicidal India ink mounts (India ink, 15 ml.; solution of Merthiolate [Lilly] 1:1000, 30 ml.; Tween-80 1:100 solution, 0.1 ml.; filtered before use). Intracellular neutral fat inclusions were stained in moist mount with a 75 per cent ethanol-acetone saturated solution of Sudan IV.

Biochemical studies. Glutamic oxalacetic transaminase was determined by a modification of the method of P. Cabaud *et al.*[12] Ammonia-nitrogen

*Thiamine hydrochloride was obtained from Hoffmann-La Roche, Inc., Nutley, N. J.; 4-methyl-5 β-hydroxyethyl thiazole, pyrimidine, and L-proline from Merck & Co., West Point, Pa.; glutamic acid and D-glucosamine hydrochloride from Eastman Kodak Co., Rochester, N. Y.; sodium glutamate and disodium salt of α-ketoglutaric acid from Bios Laboratories, New York, N. Y.

levels of cryptococcal, cell-free extracts, and spinal fluid were determined by the method of D. Seligson and K. Hirahara.[13] Keto acids, including α-ketoglutaric acid, were detected by an ascending two-dimensional paper chromatographic separation technique, using water-saturated phenol and *n*-butanol-propionic acid-water as developing solvents, followed by spray with an ethanolic solution of ninhydrin and 2,6, lutidine for detection of spots, as described by R. Rabson and N. E. Tolbert.[14] Oxalacetic, pyruvic, and α-ketoglutaric acids fluoresced yellow under ultraviolet light at 3660 Å, while β-keto acids were negative. Alpha-ketoglutaric acid migrates to a point near glutamic acid in this system with an R_f of 0.2 in solvent 1 and 0.3 in solvent 2.[14]

Vitamin Requirements of C. Neoformans

Water-soluble vitamins were sterilized by filtration through sintered-glass filters and were added aseptically, both singly and in combinations, in 100 μg. amounts to 10 ml. volumes of vitamin-free, 1 per cent glucose-BSM. The vitamins were thiamine (B_1), thiamine thiazole (4-methyl-5 β-hydroxyethyl thiazole) and thiamine pyrimidine (2-methyl-5-ethoxy-methyl-6-amino pyrimidine), riboflavin (B_2), nicotinic acid, pyridoxine (B_6), calcium pantothenate, vitamin B_{12}, biotin, choline, folic acid, inositol, L-ascorbic acid, and para-aminobenzoic acid. Seeded tubes were incubated for 30 days at 37° C. in quiescent culture.

Thiamine and its two components, thiazole and pyrimidine, were found to be growth-stimulatory for *C. neoformans*, while none of the other water-soluble vitamins cited, added either singly[15] or in combination, was stimulatory to growth. A complete mixture of water-soluble vitamins, including thiamine, furnished no greater increase in turbidity of glucose-BSM than did thiamine alone. Three human strains of *C. neoformans* and two isolated from pigeon excreta possessed a similar absolute requirement for thiamine. The results with thiamine agreed substantially with those of J. D. Reid[16] and E. G. Schmidt and his co-workers.[17] The half-maximal value of thiamine was found to be 0.0002 μg./ml. when assayed in vitamin-free, 1 per cent glucose BSM without amino acids, using vitamin-depleted cells of *C. neoformans*, and incubated 14 days at 37° C. (FIGURE 1). Rapid growth of *C. neoformans* took place over the thiamine range of 0.00001 to 0.1 μg./ml. The crest of the thiamine growth curve was at 1000 mμg./ml. (1 μg./ml.) and the optimal range was 100 to 1000 mμg./ml. (0.1 to 1 μg./ml). India ink mounts revealed that the size of capsules of *C. neoformans* increased as the thiamine content increased from 0.00001 to 0.1 μg./ml. and decreased slightly at levels higher than 1 μg./ml. A similar effect was observed when maltose and sucrose were substituted for glucose (FIGURE 1). The stimulatory effect of thiamine on the synthesis of carbohydrate capsular substance by *C. neoformans* undoubtedly reflects the primary role of thiamine as a regulator of plant carbohydrate metabolism, as thiamine

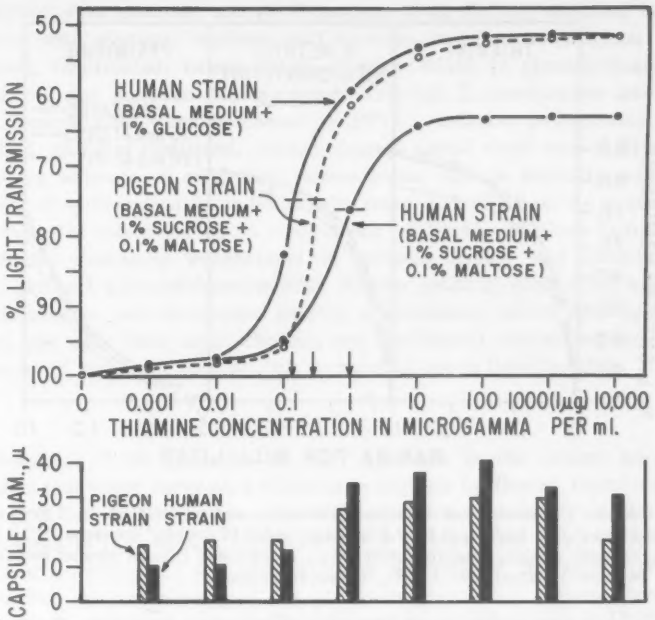

FIGURE 1. Effect of thiamine on growth and capsule synthesis of vitamin-depleted strains of *C. neoformans*, in vitamin-free basal salt medium measured after 30 days' incubation at 37° C., quiescent culture. Half-maximal value of thiamine is 0.2 mμg./ml. (0.0002 μg./ml.).

pyrophosphate (cocarboxylase). When 1 per cent glucose-BSM, enriched with 1 μg./ml. thiamine was separately seeded with 20 human isolates of *C. neoformans*, only 16 strains synthesized abundant capsular substance *in vitro*. The four poorly encapsulated strains were still capable of forming wide refractile capsules in mouse brain *in vivo*. It was therefore concluded that some additional factor besides thiamine was required for the *in vitro* encapsulation of all strains.

Heat stability of thiamine was determined by comparing the growth response of *C. neoformans* in autoclaved 1 per cent glucose-thiamine-BSM (*p*H 7.0), to that produced in media with unheated, filter-sterilized thiamine. As illustrated in FIGURE 2, autoclaving at 121° C. for 15 min. did not diminish the growth-stimulating properties of thiamine, thiazole, or pyrimidine for *C. neoformans*. Thiamine, in a concentration of 1 μg./ml., was henceforth made a constituent of BSM before autoclaving. When *C. neoformans* was provided with thiazole at 10, 1, and 0.1 μg./ml. levels, the growth response was equal to that of thiamine (FIGURE 2). The organism is therefore capable of synthesizing the pyrimidine moiety

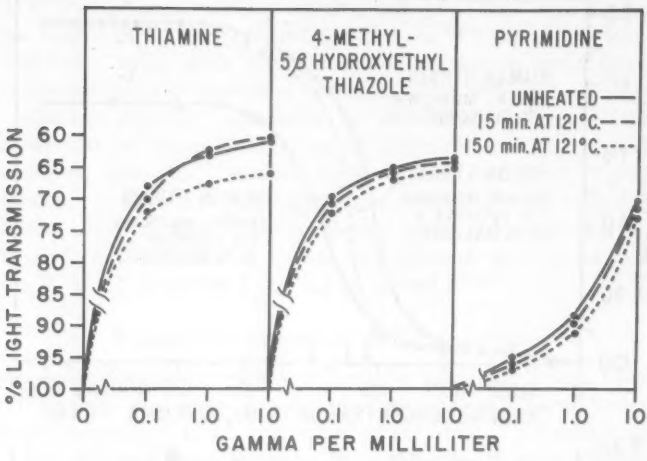

FIGURE 2. Stability of thiamine, thiazole, and pyrimidine in 1 per cent glucose-basal salt medium at *p*H 7.0 to steam under 15 pounds' pressure (121° C.). Assayed with vitamin-depleted cells of *C. neoformans* (human strain) measured after 14 days' incubation at 37° C., quiescent culture.

of thiamine and combining it with thiazole to form thiamine. *C. neoformans* is less efficient in synthesizing thiazole than pyrimidine, since considerably more of the latter must be supplied to produce a growth response equivalent to that of thiamine. In employing *C. neoformans* for the microbiological assay of thiamine over the range of 0.00001 to 0.1 μg./ml., a microbiological distinction between thiamine and thiazole would not be made by the organism and pyrimidine would not be detected.

Effect of pH, Temperature, Anaerobiosis, and Other Factors upon Capsule Size

Three human strains of *C. neoformans* and four strains isolated from pigeon excreta were nonencapsulated at *p*H 4.0 in HCl-adjusted, 1 per cent glucose-thiamine-BSM, but formed well-demarcated capsules in the same substrate at neutrality. Only minor differences in capsule sizes were noted in culture media in which the initial *p*H was raised in 0.1 increments from *p*H 6.8 to 8.0. Growth was more rapid and capsules were larger at 37° C. than at lower temperatures.

Capsule size was unaffected in thiamine-BSM by increasing the glucose content from 1 to 10 per cent. No increase in capsule size occurred when the organism was cultivated in the following media: (1) vitamin-free Yeast Base (Difco) enriched with glucose and thiamine; (2) Yeast Nitrogen Base (Difco) enriched with glucose; (3) Brewer's thioglycollate broth

enriched with glucose; and (4) Penassay Broth (Difco) enriched separately with glucose, maltose, and sucrose, and in combinations with pooled, inactivated, human serum. Heparin added to glucose-thiamine-BSM did not increase capsule size. Although *C. neoformans* retained its capsule size when incubated at 37° C., cellular proliferation was limited in filter-sterilized, normal, human spinal fluid and in filtered aqueous extracts of emulsified mouse brain. Visible turbidity was not produced by the organism in these substrates. The effect on the synthesis of capsular substance by *C. neoformans* of lipids and their hydrolytic products, commonly encountered in nervous tissue, was ascertained in 1 per cent glucose-thiamine-BSM. Neither lecithin, oleic acid, stearic acid, choline, nor cholesterol exerted a stimulatory effect upon capsule size, nor were oleic acid, choline, and cholesterol assimilated as sole sources of carbon at 1000 μg./ml. concentrations in thiamine-BSM.

Amino Acid Requirements

Because of the presence of many nutrients in the central nervous system that might serve as a stimulus to capsule synthesis, the chemical nature of the capsular substance of *C. neoformans* was closely scrutinized.

Studies of the capsule indicated that it is composed primarily of two polysaccharides: (1) an iodine-staining amylose of the starch group, described as an unbranched 1,4 glucosan, containing a preponderance of α-links, which is liberated by the cell in an acid medium,[18-20] and (2) a noniodine-staining, soluble, serologically active pentosan of the hemicellulose group, not unlike gum arabic, which is liberated into a neutral medium.[18-23] Classification of *C. neoformans* into serotypes A, B, and C[24] is based on fine differences in the capsular pentosan, the acid hydrolyzed products of which have been identified as xylose, mannose, galactose, and glucuronic acid by paper chromatographic analysis[25-27] and by serologic cross reaction.[28] Chemical tests of the cryptococcal pentosan were negative for protein, amino acid, starch, glycogen, and glucosamine.[27,29]

If *C. neoformans* could synthesize its capsular polysaccharides from a single nutrient present in the central nervous system, and from the same source synthesize its cellular amino acids, proteins, carbohydrates, fats, and enzymes, then it would be necessary for that nutrient to be strategically situated biochemically with respect to the biosynthetic pathways of nitrogen, carbohydrate, and fat metabolism. A compound satisfying many of these requirements is glutamic acid, a dicarboxylic amino acid that is an intermediate in nitrogen metabolism. It participates in transamination reactions and is therefore involved in the biological synthesis of amino acids. It undergoes reversible oxidative deamination to α-ketoglutaric acid, an important intermediate in the tricarboxylic acid cycle, and is also capable of decarboxylation to γ-aminobutyric acid.

Carbon-depleted cells of *C. neoformans* (human strain) in thiamine-BSM with $(NH_4)_2SO_4$ as sole source of nitrogen were observed to utilize the DL-, L- and D-forms of glutamic acid, L-glutamine, and sodium glutamate as sole carbon sources, and to produce wide capsules as well as intracellular inclusions of neutral fat. The assimilation of sodium glutamate is illustrated in FIGURE 3. Although growth was slow, capsules were of the same size at all glutamate concentrations up to 10 mg./ml. A growth- and capsule-inhibiting effect was observed at concentrations of glutamate in excess of 10 mg./ml. One strain of *C. neoformans* isolated from pigeon excreta produced identical growth curves in sodium glutamate-thiamine-BSM.

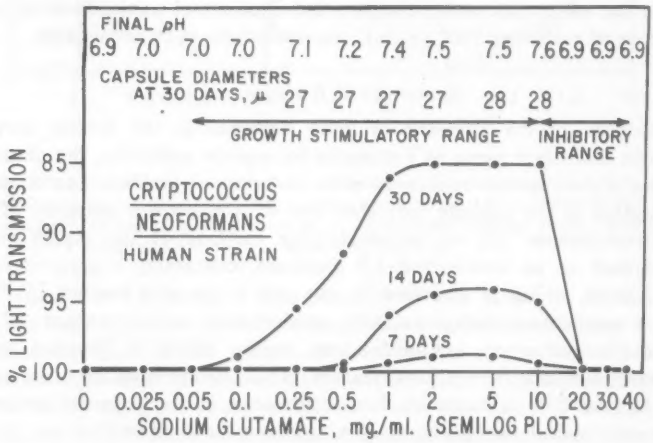
FINAL pH
6.9 7.0 7.0 7.0 7.1 7.2 7.4 7.5 7.5 7.6 6.9 6.9 6.9
CAPSULE DIAMETERS AT 30 DAYS, μ 27 27 27 27 28 28
GROWTH STIMULATORY RANGE
INHIBITORY RANGE
% LIGHT TRANSMISSION
85
90
95
100
CRYPTOCOCCUS NEOFORMANS HUMAN STRAIN
30 DAYS
14 DAYS
7 DAYS
0 0.025 0.05 0.1 0.25 0.5 1 2 5 10 20 30 40
SODIUM GLUTAMATE, mg./ml. (SEMILOG PLOT)

FIGURE 3. Utilization of sodium glutamate as sole source of carbon by *C. neoformans* (human strain) in thiamine-basal salt medium, incubated at 37° C., quiescent culture.

Cell-free filtrates of the 5 mg./ml. glutamate tubes contained 0.63 mg. ammonia nitrogen per ml., while filtrates of the glutamate-free control contained 0.51 mg./ml. Increase in the concentration of glutamate was accompanied by an increase in final *p*H and an increase in amount of ammonia nitrogen (FIGURE 3), and both were attributed to increased microbial deamination of glutamate. Serum glutamic oxalacetic transaminase levels were 0, 15, and 15 units/ml. at glutamate concentrations of 0, 0.5 and 5 mg./ml. after 30 days' incubation at 37° C. Paper chromatographic analysis of cell-free filtrates of thiamine-BSM containing 5 mg./ml. sodium glutamate, after 30 days' incubation, did not contain free α-ketoglutaric, pyruvic, and oxalacetic acids. Upon chromatographic analysis of cell-free filtrates of the same basal medium with L-proline as the sole carbon source, after 30 days' incubation no free amino acids

other than proline were detected with ninhydrin spray. Ammonia-nitrogen content of a normal human spinal fluid was 0.3 μg./ml., while that obtained from a patient with cryptococcal meningitis was approximately the same, that is, 0.35 μg./ml.

After the discovery of the ready acceptance by *C. neoformans* of glutamic acid as sole source of carbon, a review of the literature revealed that glutamic acid and its amide, glutamine, actually comprised a large part of the amino acids of the spinal fluid (TABLE 1).[30-34] All anatom-

TABLE 1

COMPOSITION OF NORMAL HUMAN CEREBROSPINAL FLUID

Component	*μg./ml.*	*Component*	*μg./ml.*
Amino acids[32]		*Carbohydrates*	
Aspartic acid	1.5	Reducing substances† (including glucose)[41]	670
Alanine	1.8		
Arginine	1.2	Fructose[42]	38
α-Aminobutyric acid	0.2	Glucosamine[42]	11
Cystine	0*	Glucuronic acid[43]	Present
Glycine	1.4	Inositol[44]	29
Glutamic acid	1.4	Mannose[45]	Present
Glutamine	3.0	Galactose[45]	Present
γ-Aminobutyric acid	0.2	Pentose, unidentified[42]	Present
Histidine	0*	Fucose[45]	Present
Lysine	1.9	α-Ketoglutaric acid[43]	0.7
Leucine	1.2	α-Oxoglutaric acid[46]	0.2
Phenylalanine	1.8	Pyruvic acid[43]	1.7
Proline	0*	Ascorbic acid[33]	6
Serine	2.5	Citric acid[33]	0.4
Tyrosine	0.8	Lactic acid[33]	170
Threonine	1.4		
Valine	1.0		
Proteins[33] *and nitrogenous compounds*		*Elements*[33]	
		Al, Ba, B, Ca, Cu, I, Mg, P, K, Na, Sr, S, Fe, HCO_3^-, Cl^-	
Albumin	550		
Fibrinogen	0		
α-Globulin	100		
β-Globulin	120		
γ-Globulin	110	*Physical properties*[33]	
Nonprotein N	190	Specific gravity	1.007
Creatinine	12	*p*H	7.4
Urea	117	CO_2 vol. %	59

*Present in abnormal spinal fluids.

†Twenty-five per cent is nonsugar.

ical divisions of the brain, furthermore, were reported to contain a considerable quantity of free L-glutamic acid (2.51 to 3.45 mg./ml. of moist tissue).[35] The adult spinal cord was reported to be rich in L-glutamic acid with an average content of 4.75 mg./ml. of moist tissue.[35] Coincidentally, these are optimal concentrations for *C. neoformans* (FIGURES 3 and 4).

Thirty-one amino acids and derivatives in concentration of 1000 μg./ml. were now added as sole sources of carbon to thiamine-BSM. The medium was seeded with carbon-depleted cells of *C. neoformans* (human strain), incubated in quiescent culture at 37° C., and observed at 7, 14, and 30 days. The results shown in TABLES 2 and 3 demonstrate ready utili-

TABLE 2

ASSIMILATION OF AMINO ACIDS AND DERIVATIVES AS SOLE SOURCES OF CARBON BY *C. NEOFORMANS* (HUMAN STRAIN), IN 1000 μG./ML. CONCENTRATIONS IN THIAMINE-BASAL SALT MEDIUM. 30 DAYS' QUIESCENT INCUBATION AT 37° C.

Compounds assimilated	*Average diameter of capsule, μ*			*Relative value of compound as source of capsular substance, expressed in per cent**			*Intracellular neutral fat inclusions (Sudan IV)*
	7d.	*14d.*	*30d.*	*7d.*	*14d.*	*30d.*	*30d.*
D-Glutamic acid	27	27	28	0	14.1	37.5	+
Sodium glutamate	27	27	29	2.6	13.2	38.8	+
L-Glutamine	ng†	ng	24	0	0	2.3	+
L-Proline	11	15	21	1.1	11.5	28.0	+
DL-Serine	ng	ng	27	0	0	5.2	+
L-Asparagine	ng	ng	24	0	0	4.6	+

*Optical density of tube times capsule diameter, compared with that of mannose in 30 days' incubation at 37° C.

† No growth.

zation of D-glutamic acid and L-proline as the sole carbon sources, and minor assimilation of DL-serine and L-asparagine. Intracellular neutral fat inclusions, stainable with Sudan IV, were synthesized by the organism from D-glutamic acid, L-glutamine, sodium glutamate, L-proline, DL-serine, and L-asparagine. Twenty-five amino acids and derivatives, listed in TABLE 3, were not assimilated as sole sources of carbon. Since L-proline appears only in abnormal spinal fluid,[32] the only amino acid of normal spinal fluid available as a nutrient and utilizable by *C. neoformans* as sole source of carbon is glutamic acid. This amino acid and its amide, glutamine, are present in the spinal fluid in relatively high concentrations.

The effect of glucose on the assimilation of sodium glutamate by *C. neoformans* (human strain) was determined in thiamine-BSM by adding

TABLE 3

AMINO ACIDS AND DERIVATIVES NOT ASSIMILATED BY *C. NEOFORMANS* (HUMAN STRAIN) AS SOLE SOURCES OF CARBON IN THIAMINE-BASAL SALT MEDIUM

DL-Alanine monohydrochloride	DL-Isoleucine
DL-Amino-*n*-caproic acid	DL-Leucine
D-Arginine	DL-Lysine dihydrochloride
DL-Aspartic acid	DL-Methionine
Cysteine HCl	DL-Ornithine
L-Cystine	DL-Phenylalanine
Glycine	Sarcosine HCl
Glycyl-glycine	DL-Threonine
Glutathione	β-2-Thienylalanine
L-Histidine dihydrochloride	DL-Tryptophan
DL-Homocysteine	L-Tyrosine
DL-Allo δ hydroxylysine HCl	DL-Valine
L-Hydroxyproline	

0.06 per cent glucose, the equivalent concentration of reducing sugar in the spinal fluid (60 mg. per cent). Sodium glutamate was noted to be growth-stimulatory at 1 to 10 mg./ml. (FIGURE 4), and to be growth-inhibitory at higher concentrations (see also FIGURE 3). The failure

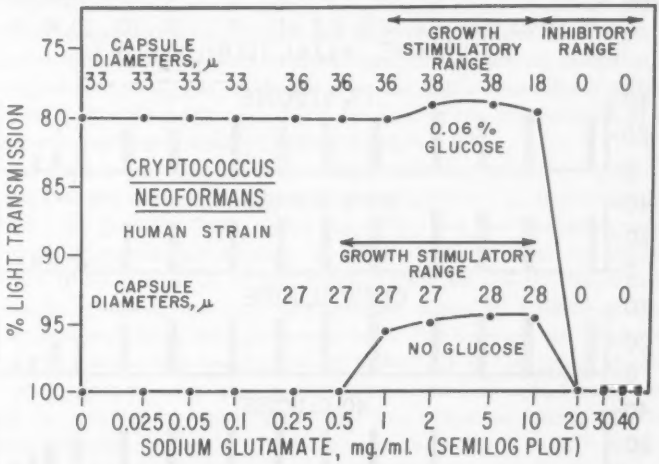

FIGURE 4. Effect of sodium glutamate on growth and capsule synthesis of *C. neoformans* (human strain) in the presence and absence of 0.06 per cent glucose (60 mg. per cent) in thiamine-basal salt medium measured after 14 days' incubation at 37° C., quiescent culture.

of glucose, a readily assimilable metabolite, to reverse the growth-inhibitory and capsule-restricting effect of overconcentrations of sodium glutamate suggests a binding effect of the latter compound on some essential enzymatic process.

The effect of glucose on the assimilation of L-glutamine by *C. neoformans* in thiamine-BSM is presented in FIGURE 5. Although density of growth was greater in the presence of glucose over the entire range of glutamine concentrations, capsule synthesis diminished sharply when glutamine exceeded 10 mg./ml. even in the presence of glucose.

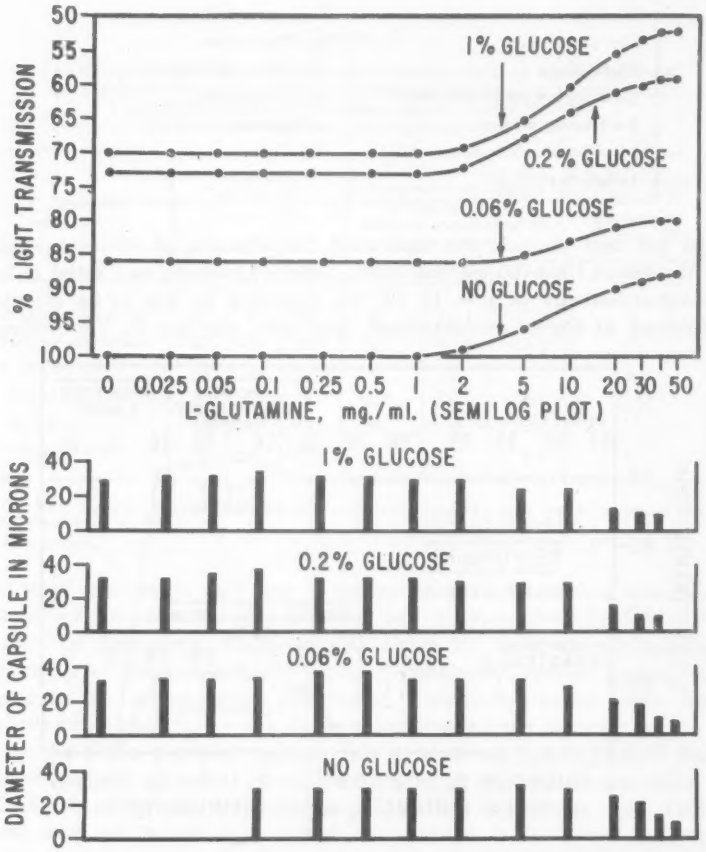

FIGURE 5. Effect of glucose and L-glutamine on the growth and capsule synthesis of *C. neoformans* (human strain) in thiamine-basal salt medium measured after 14 days' incubation at 37° C., quiescent culture.

Assimilation of α-ketoglutaric acid and sodium glutamate by *C. neoformans* was accompanied by an increase in alkalinity. Assimilation of the latter compound in the presence of 0.06 per cent glucose was also accompanied by a *p*H rise. Although an acid reaction appeared when glucose was incorporated with L-glutamine, assimilation of the latter substance was also accompanied by a perceptible *p*H rise. Although the cause for the inhibitory effect on capsule synthesis of overconcentrations of the glutamate compounds is not clear, it is not due to *p*H change, since capsule inhibition took place at widely different *p*H values, that is *p*H 4.4 and 7.1.

Carbohydrate Requirements

C. neoformans is not considered to be a fermentative yeast. It produces acid slowly from glucose, fructose, mannose, and sucrose in peptone culture medium, and does not liberate gaseous by-products, such as CO_2 or H_2. Assimilation of carbohydrates as sole sources of carbon in a synthetic medium, rather than fermentation tests, therefore has assumed greater taxonomic value in the identification of the species. *C. neoformans* is differentiated from other yeasts by its ability to grow at 37° C. and its production of urease.[1,36,37] It is differentiated from other species of the genus *Cryptococcus* by: (1) its virulence for the white Swiss mouse; (2) production of wide capsules in mouse brain upon intracerebral injection; (3) positive assimilation of glucose, maltose, sucrose, and galactose as sole carbon sources in synthetic vitamin medium; (4) failure of lactose assimilation; and (5) the negative assimilation of KNO_3 in an appropriate medium. As noted previously, *C. neoformans* is further differentiated into serotypes A, B, and C on the basis of serologic differences in the pentosan of the capsule.[24] Studies on the assimilation of carbohydrates have been limited to those cited.[1,38-40]

In TABLE 4 is listed a wide variety of mono-, oligo-, and polysaccharides that are utilized as sole sources of carbon by *C. neoformans* (human strain) in thiamine-BSM, containing $(NH_4)_2SO_4$ as the sole source of nitrogen. Interestingly enough, all of the carbohydrates and derivatives known to be present in normal spinal fluid were readily assimilated, for example, glucose, fructose, glucosamine, mannose, galactose, inositol, α-ketoglutaric acid, and glucuronic acid. Furthermore, all of the carbohydrates recoverable as products of hydrolysis of the capsular pentosan of the organism were also readily assimilated as sole carbon sources, that is, xylose, mannose, galactose, and glucuronic acid. The largest capsules were formed by *C. neoformans* in 30 days at 37° C. in ethyl alcohol and inositol, followed in order by soluble starch, dextrin, galactose, xylose, mannose, and glucose. The most abundant capsular substance was produced in 30 days in mannose, followed, in order, by glucose, fructose, xylose, sucrose, maltose, and starch. This was estimated by multiplying the optical density of each tube by the capsular

TABLE 4

ASSIMILATION OF CARBOHYDRATES AND DERIVATIVES AS SOLE SOURCES OF CARBON BY *C. NEOFORMANS* (HUMAN STRAIN) IN 1000 μG./ML. CONCENTRATIONS IN THIAMINE-BASAL SALT MEDIUM AT 37° C., QUIESCENT INCUBATION

Compound	*C atoms*	*Nature*	*Capsule diameter, μ*			*Final pH*	*Relative value of compound as source of capsular substance, expressed in per cent**			*Intracellular neutral fat inclusions (Sudan IV)*
			7d.	*14d.*	*30d.*	*30d.*	*7d.*	*14d.*	*30d.*	*30d.*
Ethyl alcohol	2	Alcohol	ng.	29	40	6.6	0	3	16	+
DL-Glyceraldehyde	3	Triose	8	26	29	5.2	0	23	35	+
α-Ketoglutaric acid‡	4	Keto acid	ng.	22	22	6.6	0	4	9	+
D-Ribose	5	Pentose	ng.	26	29	6.4	0	3	23	+
D-Xylose†	5	Pentose	19	19	32	6.2	7	34	84	+
D-Galactose†‡	6	Aldohexose	24	27	34	6.2	2	25	60	+
D-Fructose‡	6	Ketohexose	24	24	24	4.4	19	48	89	+
D-Glucose‡	6	Aldohexose	16	18	32	6.1	11	39	93	+
D-Mannose†‡	6	Aldohexose	19	19	32	6.2	21	50	100	+
D-Glucuronolactone†‡	6	Anhydride glucuronic acid	6	11	16	5.8	2	20	46	+
Inositol‡	6	Hexahydrocyclohexane	21	29	40	6.4	0	17	53	+
D-Glucosamine‡	6	Aminosugar	6	14	18	5.8	2	16	51	+
Cellobiose	12	Disaccharide (β-glucoside)	19	22	32	6.5	0	0	3	+
Maltose	12	Disaccharide (α-glucoside)	29	29	29	4.4	14	32	69	+
Trehalose	12	Disaccharide (α-glucoside)	26	29	29	4.3	0	17	61	+
Sucrose	12	Disaccharide	24	27	32	6.2	14	39	80	+
Dextrin	$(6)_x$	Polysaccharide	24	34	34	5.8	0	13	56	+
Starch, soluble	$(6)_x$	Polysaccharide	22	38	38	6.2	0	15	68	+

*Optical density of tube times capsule diameter and compared with that of mannose after 30 days' incubation at 37° C.
†Hydrolytic products of capsular pentosan of *C. neoformans*.
‡Present in normal CSF.
Symbol: ng. = no growth.

diameter of the organism. At 7 days' incubation at 37° C., capsular substance was produced most rapidly from mannose, fructose, maltose, sucrose, and glucose. In most instances, the largest capsules were found in carbohydrate-containing thiamine-BSM in which the final *p*H did not fall below 6.0.

The superiority of assimilation tests over fermentation tests for the identification of a yeast such as *C. neoformans* is evidenced by the fact that, of the 18 carbohydrate sources employed for assimilation, a frankly acid reaction was produced in 30 days with only 7 carbohydrates. Eleven of these readily assimilated carbon sources would therefore have been recorded as "slight" or "negative" on a fermentation test.

Carbohydrate intermediates not utilized in thiamine-BSM were β-glycerophosphoric acid (disodium salt), glucose-*1*-phosphate (Cori ester) in the presence of adenosine triphosphate (ATP), and glutaric acid. The highly branched polysaccharide, glycogen, was not assimilated by *C. neoformans*, while dextrin and soluble starch, both containing unbranched glucose units in 1,4 glycoside bonds, were readily assimilated and stimulated the synthesis of large capsules. The rare disaccharide, cellobiose, a glucose-β-glucoside with a 1,6 linkage, was assimilated poorly, and lactose, a β-D-galactoside, was not assimilated at all. On the other hand, the α-glucosides, trehalose and maltose, were rapidly utilized. The organism thus appears to have a preference for unbranched polysaccharides and for disaccharides containing an α-glucoside structure. The biochemical versatility of the organism is illustrated by its ability to synthesize neutral fat from all of the carbohydrate sources assimilated (TABLE 4).

Thiamine assay of spinal fluid with C. neoformans. The medium employed for the microbiological assay of thiamine consisted of thiamine-free BSM at *p*H 7.0 enriched with 0.2 per cent sodium glutamate and 1 per cent glucose and distributed in 10 ml. amounts to aluminum-capped, matched test tubes. Standard thiamine and untreated spinal fluids were added in decreasing quantities in duplicate, and tubes were autoclaved at 118° C. for 12 min. Cooled tubes were seeded with one drop each (0.07 ml.) of vitamin-depleted cells of *C. neoformans* suspended in sterile assay medium. Turbidimetric growth responses were read photometrically after tubes had been incubated in quiescent culture for 14 days at 37° C.

The standard curve for thiamine covered the range 0.00001 to 0.1 μg./ml. (FIGURE 6). The half-maximal value of thiamine was 0.0003 μg./ml. Assay of the vitamin in the same culture medium, but without glutamate, was noted previously to give a half-maximal value of 0.0002 μg./ml. (FIGURE 1). The contents of free, but not total, thiamine of 2 normal human cerebrospinal fluids were 0.0007 and 0.0010 μg./ml., while those obtained from two cases of cryptococcal meningitis were similar, that is, 0.0006 and 0.0012 μg./ml. (FIGURE 6). H. M. Sinclair[48] noted that the total thiamine content of spinal fluid varied from 0 to 0.013

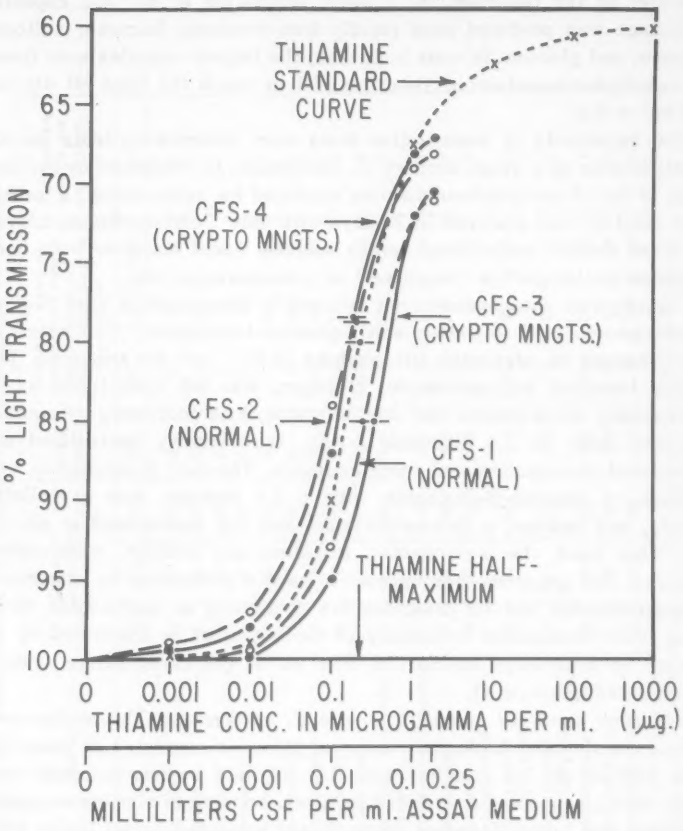

FIGURE 6. Thiamine assay of cerebrospinal fluid with vitamin-depleted cells of *C. neoformans* (human strain) in thiamine-free, basal salt medium containing 1 per cent glucose and 0.2 per cent sodium glutamate at pH 7.0 measured after 14 days' incubation at 37° C., quiescent culture. Half-maximal value of thiamine standard curve = 0.0003 μg./ml. Content of free thiamine in normal spinal fluid (CSF 1 and 2) = 0.0007 and 0.0010 μg./ml.; cryptococcal meningitis spinal fluid (CSF 3 and 4) = 0.0006 and 0.0012 μg./ml.

μg./ml. when assayed with *P. blakesleeanus*, and R. Abderhalden and K. H. Elsaesser[49] observed that total thiamine value of normal and pathological spinal fluid varied from 0.002 to 0.015 μg./ml.

The total thiamine content of the spinal fluid can be raised sharply by administration of the vitamin, according to R. Abderhalden and K. H. Elsaesser,[49] who noted an increase to 0.25 μg./ml. in the spinal fluid several hours after intravenous and intramuscular injection of 50 mg. of thiamine; the content fell to normal values within 24 to 36 hours. These

data indicate that it would be unwise to administer thiamine or vitamin B complex to patients with cryptococcal meningitis.

During studies of vitamin requirements of *C. neoformans*, it became obvious that, despite its pathogenic nature, the organism was highly suitable for the microbiological assay of thiamine. Some of its advantages as an assay organism were noted to be: (1) the absolute requirement of the organism for thiamine and its moieties and its lack of response to all other known water-soluble vitamins; (2) the high degree of sensitivity of the organism to thiamine, the half-maximal value of which was 0.0002 μg./ml. in 1 per cent glucose-BSM and 0.0003 μg./ml. in glucose-sodium glutamate-BSM; (3) the ready assimilation of glutamic acid and L-proline and weak assimilation of DL-serine and L-asparagine of a total of 24 amino acids, which reduces the likelihood of amino acid interference in an assay procedure; (4) the relatively simple synthetic substrate needed, compared with other species of microorganisms that require multiple vitamins and more complex medium; (5) the ease of preparation of smooth suspensions of vitamin-depleted cells of *C. neoformans* in standard, reproducible inocula; and (6) the ease with which periodic photometric measurements can be made, as compared with the more laborious, mycelial-weighing procedures necessary in thiamine assays with filamentous mold species, such as *P. blakesleeanus*.[47]

Cryptococcus capsule agar and capsule broth. In view of the known requirements of *C. neoformans* for vitamins, amino acids, and carbohydrates, and of the effects of these metabolites upon capsule synthesis, a synthetic capsule culture medium was readily formulated. Ammonium sulfate was provided in the formula as a source of nitrogen; sodium glutamate, maltose, and sucrose were provided as sources of carbon; mineral salts were included; and thiamine was added in optimal concentration (TABLE 5). Because of the constancy of their assimilation by all strains of *C. neoformans*, the carbohydrates of the capsule medium were limited to the disaccharides, maltose, and sucrose.

Forty strains of *C. neoformans* of diverse origins were subcultured to: (1) *Cryptococcus* capsule agar slant and broth; (2) yeast extract agar slant; and (3) Sabouraud's dextrose agar slant enriched with thiamine and biotin. All tubes were incubated at 37° C. India ink examination of the growth was made at 2, 5, 14, and 30 days. Without exception, every strain of *C. neoformans* cultivated on *Cryptococcus* capsule agar and broth showed a high percentage of encapsulated cells with wide capsules, while many strains on yeast extract and on Sabouraud's dextrose agars produced only an occasionally encapsulated cell with considerably smaller capsules (FIGURE 7).

Discussion

Infection of the central nervous system of man with *C. neoformans* is an example of exquisite parasitism that occurs when the exact nutritional

TABLE 5

CRYPTOCOCCUS CAPSULE MEDIUM

Constituent	*gm./l.*	*Constituent*	*gm./l.*
KH_2PO_4	2	Thiamine	0.001
$(NH_4)_2SO_4$	2	Maltose	5
$MgCl_2 \cdot 6H_2O$	0.2	Sucrose	5
$CaCl_2 \cdot 2H_2O$	0.02	Sodium glutamate	2
$FeCl_2 \cdot 4H_2O$	0.04	Washed agar*	20
$MnCl_2 \cdot 4H_2O$	0.0015	Double-distilled water	1000 ml.
$Na_2MoO_4 \cdot 2H_2O$	0.0015	*p*H (adjusted with 1N NaOH)	7.0
Distribute and autoclave at 118° C. for 12 min.			

*Omit for capsule broth.

needs of a parasite are supplied by the host tissue. For example, the only water-soluble vitamin required in a synthetic medium by *C. neoformans* is thiamine, the half-maximal growth value being 0.0002 μg./ml., while the free thiamine content of normal and abnormal spinal fluid is 0.001 μg./ml. In addition, the amino acids mainly utilized as sole sources of carbon by *C. neoformans* are glutamic acid and proline, while glutamic acid and glutamine form the largest part of the amino-nitrogen of the spinal fluid, and proline is present in only abnormal spinal fluids. Carbohydrates normally present in spinal fluid are glucose, fructose, galactose, glucosamine, glucuronic acid, inositol, mannose, and α-ketoglutaric acid; *C. neoformans* is capable of rapidly assimilating every one of these carbohydrates as sole sources of carbon. Ammonia and urea are present in spinal fluid; both substances are readily assimilated as sole source of nitrogen by the organism, and all strains of *C. neoformans* are capable of hydrolytic cleavage of urea by virtue of their rapid liberation of urease.[37] The exact mineral requirements of *C. neoformans* remain to be determined but, since the spinal fluid is rich in mineral elements (TABLE 1), many of which are necessary for the functioning of yeast enzyme systems, the mineral requirements of the organism are undoubtedly fulfilled. It is therefore concluded that the unusual affinity of *C. neoformans* for the central nervous system is due to the presence of vitamins and metabolites that adequately fill the nutritional requirements of this organism, that is, thiamine, glutamic acid and glutamine, a number of spinal fluid carbohydrates, and essential minerals.

Knowledge of the nutritional need of *C. neoformans* may provide clues to other biological phenomena. For example, the high incidence and

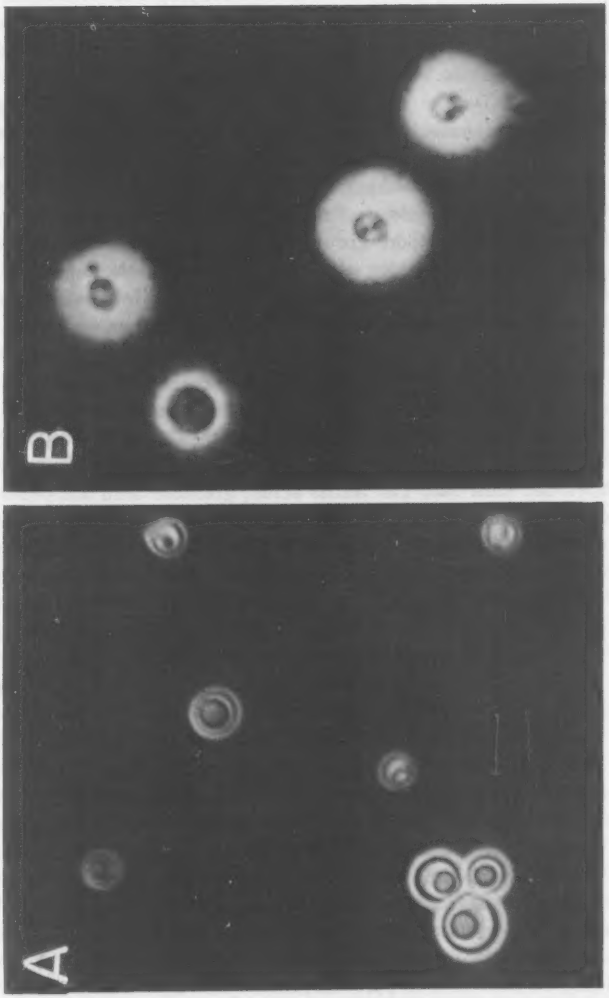

FIGURE 7. Comparison of capsule size of *C. neoformans* (human strain) cultivated at 37° C. on (a) yeast extract agar and (b) *Cryptococcus* capsule agar. India ink mount. X600.

disseminated nature of cryptococcosis in reticuloendothelial diseases such as leukemia, Hodgkin's disease, and lymphosarcoma[50] must undoubtedly be due to specific changes in the nutritional content of leukemic blood cells and plasma that favor the growth of *C. neoformans*. Since the specific vitamin, amino acid, and carbohydrate requirements of *C. neoformans* are now known, a study of these constituents in the leukemic cell and plasma may shed light upon important factors in reticuloendothelial disturbances.

In the absence of effective medications for the treatment of cryptococcal meningitis, continued search for new medications is indicated. Systematic studies of metabolite analogues of compounds that are essential for the nutrition of *C. neoformans* may reveal a clinically useful, inhibitory drug. Metabolite analogues of thiamine, however, such as neopyrithiamine, failed to support growth of *C. neoformans* and did not effectively inhibit the utilization of added thiamine by the organism.[17] The usefulness of metabolite analogues of glutamic acid for the inhibition of *C. neoformans* and other fungi is now under investigation.

Energy-yielding metabolic pathways. Judging from the ready utilization of mono-, oligo-, and polysaccharides as sole sources of carbon by *C. neoformans*, one may deduce (1) that carbohydrate substrates serve as the major source of energy required for biosynthesis by the organism, and (2) that sufficient enzyme systems are present for the formation of carbohydrates and their use. The amino acids, glutamic acid and proline, are utilized less readily than are carbohydrates, but nevertheless appear to serve as secondary sources of energy. Glutamic acid, L-proline, together with α-ketoglutaric acid form important links through which the oxidative breakdown of protein is connected with that of carbohydrate. Fatty acids such as oleic and stearic acids are not utilized by *C. neoformans* as sources of energy.

It is unlikely that glucose is degraded by way of the Embden-Meyerhof glycolytic system or the monophosphate shunt because of the organism's inability to accept the following intermediates of the system as sole carbon sources in thiamine-BSM: glucose-*1*-phosphate (Cori ester) and the sodium salt of β-glycerophosphoric acid in the presence or absence of adenosine triphosphate (ATP). The mechanism by which *C. neoformans* converts carbohydrates to smaller carbon units remains to be clarified. Evidence for the functioning of the Krebs tricarboxylic acid cycle[51,52] in the carbohydrate metabolism of *C. neoformans* may be found in the utilization by the organism of α-ketoglutaric acid. The organism also readily assimilates aldo- and ketohexoses and glyceraldehyde. The latter compound may be converted to pyruvate, from which the di- and tricarboxylic acids of Krebs cycle are known to originate. Thiamine pyrophosphate is required at two points, that is, for the oxidative decarboxylation of pyruvic acid as well as α-ketoglutaric acid. Thiamine may be required also as a transfer cofactor for α-keto acids. Although the ketonic acids,

α-ketoglutaric, oxalacetic, and pyruvic acids, which arise in the course of oxidation of citrate, were absent in cryptococcal, cell-free filtrates of glutamate-thiamine-BSM, as determined with paper chromatographic analysis, it is likely that they are produced intracellularly or converted as rapidly as formed.

Energy-consuming (biosynthetic) pathways. The starting point for synthesis of protein by *C. neoformans* appears to be ammonia as the primary source of nitrogen, in the presence of carbohydrates or amino acids as sources of carbon and energy. All three members of the glutamic acid isotope family (glutamic acid, proline, asparagine) and α-ketoglutaric acid are assimilated by *C. neoformans* as sole sources of carbon and energy. The most likely pathway, therefore, for conversion of ammonia to α-amino acids is through the ketoglutarate gateway, through the hydrogenation of an α-ketodicarboxy acid to L-glutamic acid. The presence of glutamic oxalacetic transaminase in cryptococcal, cell-free filtrates as well as the utilization of glutamic acid and α-ketoglutaric acid indicates that *C. neoformans* is capable of the biosynthesis of its natural amino acids by transamination. L. L. Uzman *et al.*[53] have shown that *C. neoformans* is capable of the synthesis of sixteen amino acids from $(NH_4)_2SO_4$ and glucose in a thiamine, mineral synthetic medium. The dicarboxylic amino acids, glutamic and aspartic acids, predominated among the acid hydrolyzed products of the somatic protein.[53] The amino acid content of cryptococcal cells grown in glutamic acid and in other carbon sources remains to be determined.

Starting points for carbohydrates of the cryptococcal cell and capsule may be any one of a wide variety of simple or complex carbohydrates, from glyceraldehyde to starch, for which the organism apparently posesses the necessary enzyme systems. The glutamic acid family (glutamic acid, proline, asparagine) serves as sole source of carbon and energy and enables the organism to synthesize carbohydrates as well as lipids, presumably through the oxidative deamination of glutamic acid to α-ketoglutaric acid. The carbohydrate building block appears to be a simple three-carbon sugar, such as the triose, glyceraldehyde, which is readily assimilated and from which the organism is able to synthesize its intracellular carbohydrates and capsular polysaccharide. As noted previously, *C. neoformans* utilizes unbranched rather than branched polysaccharides and assimilates α-glucoside disaccharides rather than β-glucosides.

Lipids of *C. neoformans* appear as small, intracellular inclusions. Judging from absorption of Sudan IV (FIGURE 8), the lipid inclusions are composed primarily of neutral fats. Starting points for lipid synthesis by *C. neoformans* are apparently furnished by a wide variety of carbohydrates as well as by the amino acids, glutamic acid, L-proline, L-asparagine, and DL-serine. *C. neoformans* does not utilize the fatty acids, oleic and stearic acids, or lecithin as sole sources of carbon, and it assimilates glycerol poorly. The pathway of lipid synthesis for *C. neo-*

formans is unknown at present. Since fat inclusions are synthesized by the organism in thiamine-BSM containing glutamic and α-ketoglutaric acids as sole carbon sources, it is likely that biosynthesis of fats from carbohydrates passes through these key intermediates.

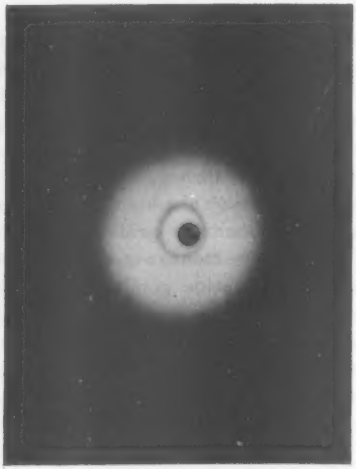

FIGURE 8. Synthesis of neutral fat from soluble starch by *C. neoformans* (human strain) in thiamine-basal salt medium at 37° C. Note darkly staining intracellular inclusion that absorbs the red color of Sudan IV stain, India ink mount. X1000.

The nuclear chromatin of *C. neoformans*, desoxyribonucleic acid (DNA), was synthesized by the organism in thiamine-BSM starting from the carbohydrates, D-ribose, D-glucose, sucrose, and soluble starch. Desoxyribonucleic acids were detected as greenish-yellow fluorescent, intracellular inclusions by fluorescent microscopy of acridine orange-stained suspensions at *p*H 4.4 in acetic acid-sodium acetate buffer, following the technique of Armstrong.[54] Cytoplasmic chromatin (RNA) inclusions were not visualized.

Summary

Studies were conducted of the vitamin, amino acid, and carbohydrate requirements of *Cryptococcus neoformans*, and their effects upon capsule synthesis in chemically defined culture media. *C. neoformans* proved to be thiamine-deficient, demonstrating an absolute requirement for thiamine and its moieties, and a lack of response to other water-soluble vitamins. In a glucose synthetic basal medium, thiamine exerted a stimulatory effect upon capsule synthesis by *C. neoformans* over the range of concentrations 0.00001 to 0.1 μg./ml. The organism was capable of syn-

thesizing thiamine pyrimidine and thiamine thiazole and of combining them into thiamine. Of the 25 amino acids furnished as sole sources of carbon in synthetic medium, the organism assimilated members of the glutamic acid family: D-, L-, and DL-glutamic acid, L-proline and, to a limited extent, L-asparagine and DL-serine. A number of mono-, oligo-, and polysaccharides, ranging from glyceraldehyde to starch, were also assimilated as sole sources of carbon by *C. neoformans*. All of the various carbohydrates that exist in normal cerebrospinal fluid were likewise assimilated. The organism has a preference for unbranched polysaccharides and for disaccharides containing α-glucoside structure. Carbohydrate intermediates of the Embden-Meyerhof glycolytic system were not assimilated, while aldo- and ketohexoses, DL-glyceraldehyde and the tricarboxylic intermediate, α-ketoglutaric acid, were readily accepted. Neutral fat inclusions were synthesized from every carbohydrate source that was assimilated. Desoxyribonucleic acid was synthesized from D-ribose, D-glucose, sucrose, and starch. Energy-yielding and energy-consuming metabolic pathways for carbohydrate, protein, and fat were provisionally indicated.

A microbiological assay technique for thiamine employing *C. neoformans* was described. Normal and cryptococcal meningitis spinal fluids contained 0.001 μg./ml. free thiamine, the half-maximal value of which was 0.0003 μg./ml. for *C. neoformans* in synthetic medium.

A chemically defined *Cryptococcus* capsule agar and broth culture medium was formulated that contained mineral salts, thiamine, sodium glutamate, maltose, and sucrose. The media were observed to stimulate *in vitro* encapsulation of all strains studied.

The unusual affinity of *C. neoformans* for the central nervous system is attributed to the availability in the brain and cerebrospinal fluid of nutrients in the optimal concentrations required by the organism. Attention was directed to a relation between the nutritional requirements of *C. neoformans* and its high incidence and disseminated nature in reticuloendothelial diseases, such as leukemia, Hodgkin's disease, and lymphosarcoma. The inadvisability of administering thiamine to patients with cryptococcal meningitis was emphasized.

Acknowledgments

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References

1. LITTMAN, M. L. & L. E. ZIMMERMAN. 1956. Cryptococcosis-Torulosis. 205 pp. Grune & Stratton. New York, N. Y.

2. GOLD, W., H. A. STOUT, J. F. PAGANO & R. DONOVICK. 1955-1956. Amphotericins A and B, antifungal antibiotics produced by a streptomycete. I. *In vitro* studies. Antibiotics Ann. : 579-586.
3. VANDEPUTTE, J., J. L. WACHTEL & E. T. STILLER. 1955-1956. Amphotericins A and B, antifungal antibiotics produced by a streptomycete. II. The isolation and properties of the crystalline amphotericins. Antibiotics Ann. : 587-591.
4. LITTMAN, M. L. 1957. Preliminary observations on the intravenous use of amphotericin B, an antifungal antibiotic, in the therapy of acute and chronic coccidioidal osteomyelitis. Proc. Symposium on Coccidioidomycosis. Public Health Service Public. No. **575** : 86-94. Phoenix, Arizona.
5. CIRCULAR. 1957. Fungizone for injection. : 1-15. Squibb Inst. Med. Research, New Brunswick, N. J.
6. LITTMAN, M. L., P. L. HOROWITZ & J. G. SWADEY. 1958. Coccidioidomycosis and its treatment with amphotericin B. Am. J. Med. **24**: 568-592.
7. HARRELL, E. R. & A. C. CURTIS. 1957. The treatment of North American blastomycosis with amphotericin B. A.M.A. Arch. Dermatol. **76**: 561-569.
8. LITTMAN, M. L. Unpublished data.
9. APPELBAUM, E. & S. SHTOKALKO. 1957. Cryptococcus meningitis arrested with amphotericin B. Ann. Internal Med. **47**: 346-351.
10. RUBIN, H., P. H. LEHAN, M. J. FITZPATRICK & M. L. FURCOLOW. 1957-1958. Amphotericin B in the treatment of cryptococcal meningitis. Antibiotics Ann. : 71-74.
11. UTZ, J. P., D. B. LOURIA, N. FEDER, C. W. EMMONS & N. B. MC CULLOUGH. 1957-1958. A report of clinical studies on the use of amphotericin B in patients with systemic fungal diseases. Antibiotics Ann. : 65-70.
12. CABAUD, P., R. LEEPER & F. WRÓBLEWSKI. 1956. Colorimetric measurement of serum glutamic oxalacetic transaminase. Am. J. Clin. Pathol. **26**: 1101-1105.
13. SELIGSON, D. & K. HIRAHARA. 1957. The measurement of ammonia in whole blood, erythrocytes and plasma. J. Lab. Clin. Med. **49**: 962-974.
14. RABSON, R. & N. E. TOLBERT. 1958. Detection of α-ketoglutaric acid and other keto-acids on paper chromatograms with ninhydrin. Nature. **181**: 50-51.
15. LITTMAN, M. L. & M. A. PISANO. Unpublished data.
16. REID, J. D. 1949. The influence of the vitamin B complex on the growth of Torulopsis *(Cryptococcus) neoformans* on a synthetic medium. J. Bacteriol. **58**: 777-782.
17. SCHMIDT, E. G., J. A. ALVAREZ-DE CHOUDENS, N. F. MC ELVAIN, J. BEARDSLEY & S. A. A. TAWAB. 1950. A microbiological study of *Cryptococcus neoformans*. Arch. Biochem. **26**: 15-24.
18. ASCHNER, M., J. MAGER & J. LEIBOWITZ. 1945. Production of extracellular starch in cultures of capsulated yeasts. Nature. **156**: 295.
19. MAGER, J. & M. ASCHNER. 1947. Biological studies on capsulated yeasts. J. Bacteriol. **53**: 283-295.
20. HEHRE, E. J., A. S. CARLSON & D. M. HAMILTON. 1949. Crystalline amylose from cultures of a pathogenic yeast *(Torula histolytica)*. J. Biol. Chem. **177**: 289-293.
21. NEILL, J. M., C. G. CASTILLO, R. H. SMITH & C. E. KAPROS. 1949. Capsular reactions and soluble antigens of *Torula histolytica* and *Sporotrichum schenckii*. J. Exptl. Med. **89**: 93-106.
22. MAGER, J. 1947. Studies on the polysaccharides of capsulated yeasts. Biochem. J. **41**: 603-609.
23. STANLEY, N. F. 1949. Polysaccharide haptens from *Torula histolytica*. Australian J. Exptl. Biol. Med. Sci. **27**: 409-415.
24. EVANS, E. E. & J. F. KESSEL. 1951. The antigenic composition of *Cryptococcus neoformans*. II. Serologic studies with the capsular polysaccharide. J. Immunol. **67**: 109-114.

25. DROUHET, E. & G. SEGRETAIN. 1950. Biologie et pouvoir pathogène de *Torulopsis neoformans (Torula histolytica)*. Rev. pathol. comparée et hyg. gén. **50**: 37-44.
26. EVANS, E. E. & J. W. MEHL. 1951. A qualitative analysis of capsular polysaccharides from *Cryptococcus neoformans* by filter paper chromatography. Science. **114**: 10-11.
27. EVANS, E. E. & R. J. THERIAULT. 1953. The antigenic composition of *Cryptococcus neoformans*. IV. The use of paper chromatography for following purification of the capsular polysaccharide. J. Bacteriol. **65**: 571-577.
28. HEIDELBERGER, M., S. A. BARKER & P. A. REBERS. 1957. Cross-reactions of anthrax and *Cryptococcus*-A polysaccharides in Type XIV antipneumococcal horse serum. Science. **125**: 748.
29. FOLEY, G. E. & L. L. UZMAN. 1952. Studies on the biology of *Cryptococcus*. IV. Isolation of a highly polymerized polysaccharide from encapsulated strains. J. Infectious Diseases. **90**: 38-43.
30. RICHTER, D., R. M. C. DAWSON & L. REES. 1949. A note on the ammonia and glutamine content of the cerebrospinal fluid. J. Mental Sci. **95**: 148-152.
31. LUDEWIG, S. 1953. Glutamine, glutamic acid, and γ-aminobutyric acid in cerebrospinal fluids. A.M.A. Arch. Neurol. Psychiat. **70**: 268-270.
32. LOGOTHETIS, J. 1955. A study of free amino acids in the human cerebrospinal fluid. Neurology. **5**: 767-776.
33. SPECTOR, W. S. 1956. Handbook of Biological Data. : 57. Saunders. Philadelphia, Pa.
34. HUISMAN, T. H. J. 1955. Onderzoek naar het gehalte aan verschillende aminozuren van de liquor cerebrospinalis. Ned. Tijdschr. Geneesk. **99**: 3357-3361.
35. HIMWICH, W. A., W. T. SULLIVAN, B. KELLEY, H. B. W. BENARON & B. TUCKER. 1955. Chemical constituents of human brain. J. Nervous Mental Disease. **122**: 441-447.
36. SEELIGER, H. P. R. 1956. Use of a urease test for the screening and identification of cryptococci. J. Bacteriol. **12**: 127-131.
37. LITTMAN, M. L. 1957. An improved method for detection of urea hydrolysis by fungi. J. Infectious Diseases. **101**: 51-61.
38. BENHAM, R. W. 1955. The genus *Cryptococcus:* The present status and criteria for the identification of species. Trans. N. Y. Acad. Sci. Ser. II. **17**(5): 418-429.
39. EMMONS, C. W. 1952. *Cryptococcus neoformans* strains from a severe outbreak of bovine mastitis. Mycopathol. et Mycol. Appl. **6**: 231-234.
40. KAO, C. J. & J. SCHWARZ. 1957. The isolation of *Cryptococcus neoformans* from pigeon nests. Am. J. Clin. Pathol. **27**: 652-663.
41. EASTHAM, M. D. & K. R. KEAY. 1952. The carbohydrates in normal and abnormal cerebrospinal fluid. J. Clin. Pathol. **5**: 319-323.
42. EASTHAM, M. D. & K. R. KEAY. 1951. The carbohydrates of human cerebrospinal fluid. Biochem. J. Proc. **50**: iv-v.
43. ZAPPI, F. & M. L. CASORATI. 1951. I. Chetoacidi del liquor umano normale. Studio cromatografico. Ricerca Sci. **21**: 372-378.
44. NIXON, D. A. 1955. Cerebrospinal fluid inositol and its rise in post-mortem specimens. J. Physiol. **129**: 272-280.
45. ROBOZ, E., J. B. MURPHY, W. C. HESS & F. M. FORSTER. 1955. Isolation and determination of the glycoproteins in cerebrospinal fluid. Proc. Soc. Exptl. Biol. Med. **89**: 691-694.
46. MCARDLE, B. 1957. The quantitative estimation of pyruvic and α-oxoglutaric acids by paper chromatography in blood, urine and cerebrospinal fluid. Biochem. J. **66**: 144-148.
47. SCHOPFER, M. W. H. 1935. Recherches sur l'emploi possible d'un test végétal pour la vitamine B^1. Essai d'étalonnage. Bull. soc. chim. biol. **17**: 1097-1109.
48. SINCLAIR, H. M. 1939. The estimation of vitamin B_1 in cerebro-spinal fluid. Biochem. J. **33**: 1816-1821.

49. ABDERHALDEN, R. & K. H. ELSAESSER. 1943. Das vorkommen von aneurin und cocarboxylase im liquor. Arch. ges. Physiol. Pflüger's. **247**: 24-33.
50. ZIMMERMAN, L. E. & H. RAPPAPORT. 1954. Occurrence of cryptococcosis in patients with malignant disease of reticuloendothelial system. Am. J. Clin. Pathol. **24**: 1050-1072.
51. KREBS, H. A. 1943. The intermediary stages in the biological oxidation of carbohydrate. Advances in Enzymol. **3**: 191-252.
52. OCHOA, S. 1954. Enzymic mechanisms in the citric acid cycle. Advances in Enzymol. **15**: 183-270.
53. UZMAN, L. L., H. ROSEN & G. E. FOLEY. 1956. Studies on the biology of *Cryptococcus*. VI. Amino acid composition of the somatic proteins of *Cryptococcus neoformans*. J. Infectious Diseases. **98**: 208-210.
54. ARMSTRONG, J. A. 1956. Histochemical differentiation of nucleic acids by means of induced fluorescence. Exptl. Cell Research. **11**: 640-643.

SECTION OF ANTHROPOLOGY

THE CHANGING ABORIGINES OF WESTERN AUSTRALIA*

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In recent years a growing amount of anthropological research throughout Australia has been devoted to the changes that have taken place among Australian aborigines. One can attribute this interest in acculturation to two factors.

(1) Indigenous aboriginal culture is fast dying out, with the result that there are only a few tribes in the very remote inland areas that still live in a traditional manner, and nearly all of these have had some contact with Europeans.

(2) The remaining aborigines and part-aborigines who live scattered throughout Australia have had intensive contact with Europeans for several generations and have become extensively acculturated. The extent of this detribalization depends largely upon the intimacy and scale of contact with Europeans. Although full-bloods have gradually decreased in numbers throughout Australia, the mixed-blood population has shown marked increases. These facts have brought about a realization on the part of the administrations handling native affairs that effective measures must be taken to ensure that these people are given educational and economic opportunities that will enable them to take their places as ordinary citizens in Australian society. An official policy of assimilation has superseded the earlier protective policies that were quite unsuited to the changing situation.

However, these earlier policies have left their impact upon the present-day people, and the task of assimilating them still presents formidable problems. Studies by anthropologists have revealed the sorts of obstacles that administrators may expect to encounter, for among many aborigines there has developed a sense of group identity that makes them resist some of these efforts at assimilation. These aborigines have accepted the idea that they are different from white people and do not need to live according to the same standards. This feeling of an aboriginal group identity seems largely to have emerged from the common experiences that these people have shared as a result of their legal and social classification as natives with an inferior status within the

*This paper, illustrated with slides, was presented at a meeting of the Section on April 28, 1958.

This article is based on field work and research done between 1955 and 1957 in the Murchison area of Western Australia. The research project was financed by the University of Western Australia, Perth, Australia, where the author was Senior Research Fellow with the Psychology Department.

white community. Their social exclusion from white groups has welded a sense of common identity among them.

Among natives of the Murchison, anyone who is colored or who lives like a native is referred to as a Jamadji (meaning black fellow) while they refer to any white person or native who lives like a white person by the term Wadjbila (white fellow). This sense of a common identity with other natives has been accentuated in recent years by the growth of communications within the state. Highways, railways, and the radio have all facilitated the link between natives, who formerly would have had very little contact with one another, in widely scattered areas of Western Australia.

Now networks of relationship have begun, and people have intermarried with persons who may have come from localities several hundreds of miles away. This intermingling has had the effect of spreading certain aboriginal traits and cultural traditions, such as songs, among widely separated groups, and it has sometimes led to a certain amount of resurgence of traditional culture in areas where the local songs and ceremonies have long been forgotten, and where songs and corroborees from more remote areas are adopted as substitutes. This process has been observed in the west Murchison, where ceremonies and songs belonging to the east Murchison (which has greater numbers of traditionally oriented natives) have spread among the station natives of this area. I was able to record many such songs. Nevertheless, aboriginal culture on the whole is fast dying out, and it is probably only a matter of years before even these surviving traits will have been discarded. One can grasp the extent of the changes that have taken place only if one takes into account some of the major phases of native-white contacts since the first settlement of Western Australia.

Demographic Changes

Scattered throughout Western Australia are about 21,000 persons who have a predominance of aboriginal blood and are therefore legally classed as natives. However, these figures can be misleading; they give little indication of the social realities that lie behind them, for the legal definition of a native is based upon racial rather than cultural criteria. Consequently, many people who are classified and treated as natives simply because they possess a preponderance of aboriginal blood may bear little cultural resemblance to aborigines. Furthermore, the full-blooded aborigines have declined in numbers ever since the establishment of the first European settlement and, while they have decreased by one third, the number of half-castes has increased more than three-fold since 1926.

Changes from Native Subsistence to a European Economy

Western Australia was not colonized until 1829, but within less than forty years the natives in the southwest of the state had greatly de-

clined in numbers and were largely detribalized. The rapidity of this process can be attributed largely to a wholesale removal of aborigines from their tribal lands that has undermined the very basis of their culture. Not only did these lands supply them with food and game; the natural landmarks of the tribal territory were incorporated in their myths and sacred ceremonies, and these became meaningless once they were deprived of their water holes and sacred sites.

The Murchison area was first explored in 1870; a few years later the first sheep were brought into the area and allowed to graze on the newly opened station properties. The aborigines, members of the Wadjeri tribe who had formerly hunted in that territory, were trained by the settlers to herd their sheep and draw water. In return they were given rations of food. The aborigine proved well suited for stock work, for his knowledge of the country and his skill in bushcraft enabled him to find sheep in areas where white men would soon become lost. He also showed remarkable aptitude for handling horses, and these qualities still make natives an indispensable part of the pastoral and cattle industries.

Records show that, by 1899, more than three-fourths of all the natives in the Murchison district extending 300 miles inland had been in service to the settlers for many years.

Since the sheep had driven out much of the natural game and eaten much of the vegetation, the natives became dependent upon the settlers for food, and they were employed under a system of indenture that bound them to remain on their station and made it a punishable offense for them to abscond. The relations developed at this time between the native and his station "boss" have persisted on many stations to the present day. Station managers regarded their aboriginal workers as a part of the property, and treated them as dependent children who could not be expected to assume individual responsibilities. Many station natives accepted this attitude, as is well illustrated in the following remarks made by a Murchison station hand: "I'd sooner be a Jamadji because white people is the boss of us and white people are there to see everything is fair.... The White man's got to be boss of him to see he don't get silly.... I'd rather not be boss – they got this country and we can't say that much."

Station natives were housed in rough bush shelters; since no wages were paid and no education was offered, they remained ignorant of the value of money and, even today, they tend to squander their earnings on nonessentials. On the other hand, they were never required to perform very hard work, for there were always more native workers and their dependents on the station than were actually needed.

These settled groups of station workers developed a loyalty to their stations, and there are some stations in the Murchison today that still employ the descendants of the first generation of workers. Even this is changing, however, as many natives leave these stations in search of higher wages and better living conditions.

Even among the first generation that settled on stations the strict rules of sectional kinship and exogamy began to break down. Instead of leaving their country in search of the proper marriage partners, young people more often sought other marriageable natives from a nearby station. These breaches of tribal law would have been punished by death or spearing under normal circumstances, but the tribal elders were powerless to prevent the white station owners from protecting their workers from injury, and station owners thus gradually undermined and superseded the authority of the old men. There was a natural tendency to discourage those customs and ceremonies that interfered with the routine of station work, but many stations permitted their natives to hold "cowerie meetings" or man-making ceremonies during the Christmas period at the height of summer, when the busy season is at an end. These meetings are still occasionally held even today on certain stations, but they have gradually lost much of their traditional significance, for the present-day "tribal" leaders know only a smattering of the traditional ceremonies. The meanings of songs and rites died in great measure with the elders, who did not hand down their knowledge. Nevertheless, the surviving rituals evoke powerful emotional sentiments among those men who have "been through the law"—those who have been circumcised, have learned the secret songs, and have been given secret boards. "Going through the law" helps to establish bonds of friendship and mutual obligation between men; even some half-castes who have come to the Murchison from more southern areas have attended these meetings, because it gives them prestige within the group: "It means I can go to different people and they all know me."

However, the survival of traditional culture on Murchison stations was due largely to their relative isolation from the towns; under the greater freedom of movement that natives have enjoyed since World War II, one finds that many of them prefer a trip to the nearest town to attend some race meeting or gambling school rather than a corroboree. As one old man stated:

"Those days before the war they'd have the Christmas meeting for the boys to put them through the law. A lot of old fellows were still around then and still carrying on the law. They've died off since and though they taught the young fellows what to do, nobody bothers now; they're all going the white way now. If you try to explain anything to young fellows now they just laugh at you. They're not afraid of the old people like they used to be one time; then they'd kill you if you didn't do what you was told."

Unemployment and Prejudice Since 1900

From the year 1900 onward a new phase in native-white relations developed. Until then the aborigine had been an indispensable source

of manpower to the settlers, particularly in the north. Efforts might well have been directed then toward absorbing the half-caste population into the general community. Instead of this gradual process of integration we find a growing antinative feeling that became increasingly evident during the years 1900 to 1920 and that led to pressure, from various groups within the white community, for the segregation of aborigines and, especially, half-castes on special reserves. Up to that time there were no special native reserves in Western Australia, but these were now established by segregating unwanted lands on the outskirts of country towns and assigning these to unemployed natives, who were not permitted to enter the towns without special permits.

The antinative feeling was due partly to the public movement that arose with the official adoption of the White Australia Policy in 1901, but it was more directly related to the increase in white population after the gold-rush period of 1890 to 1895. The population of Western Australia doubled during those years, and white men began seeking employment in country areas, replacing many of the aborigines, who were assigned the more menial tasks. At this time, a number of unfavorable stereotypes about aborigines and half-castes began to circulate; many of them persist to the present day and are used as rationalizations for the low social and economic position that natives have been forced to occupy in Western Australia. For example, it was said that natives are lazy and incapable of sustained work and that they are nomadic and unable to remain in one place because they have "an instinct to go walkabout."

Gradually unemployment became common among native groups, especially those near towns and in the farming areas. The natives would obtain work only at certain times of the year, as during the harvesting; at other times they were forced to depend upon food rations from the government. This pattern of unemployment and economic insecurity gave rise to an increase in drunkenness and minor delinquencies among natives (who are not legally permitted intoxicants). To cope with this situation and with the growing public agitation for their segregation, between the years 1905 and 1948 the Native Administration began to subject natives to rigorous control under a system of police protectors, and removed many under warrant to various government settlements. These settlements became a sort of clearing house for unwanted natives—sick, orphaned, widowed, unemployed, or delinquent. Space does not permit an enumeration of the numerous clauses in the Native Administration Act (1905 to 1948) that were applied to natives, but these restrictions and social barriers have left their mark upon all the natives who came into contact with them; many of the behavior patterns that still persist in present-day native camps in the Murchison can be directly attributed to the experiences that these people underwent during their youth in native settlements. Since 1948 there have been numerous

reforms in the administration of natives in Western Australia; most of the repressive legislation has been repealed, but the new administration faces some severe problems in attempting to assimilate people who have learned to play the role of being native, as this role was defined in the expectations of the white community regarding natives in Western Australia. For more than fifty years the white community has held differing behavioral expectations for its white and native members, and has thereby indirectly fostered a differing set of social norms in the two communities. The new policy, which seeks to merge the two, faces a formidable task in re-educating both whites and aborigines in their mutual expectations.

The "Jamadji Way" and the "White Way"

During my field work in the Murchison I conducted a number of interviews with both station natives and those living around towns in an effort to obtain the native self-image by the question: "How do the Jamadjis differ from white people?" The answers showed that most natives conceive their difference from white people mainly in terms of color because they feel that they are treated differently. As one woman put it, "The Jamadjis are really more white than native, but they're still classed as natives." Although a few surviving traits of aboriginal culture, such as words of dialect, bushcraft, and interest in the native law, do help to weld their sense of identity, it is basically their common rejection by white people and their low social and economic position that gives them identity, for there are many half-castes and full-bloods who are fully accepted within the Jamadji group despite the fact that they know nothing of traditional aboriginal culture or language.

There is also a feeling that there are certain advantages in being a Jamadji. The most commonly cited were: Jamadjis have less responsibilities than white people. They are free to come and go as they choose. They can live more easily and cheaply off the bush. They can get out of trouble more easily. The following is a summing up of the "Jamadji way" by a half-caste woman.

"I'm a Jamadji. Jamadjis are dirtier than the white fellow. They won't keep themselves clean—they don't look after themselves, they just think of walking about. If they got houses they would not keep them clean, because they're too lazy to do it. They just want to walk about on their own gambling; that would not keep a house clean. There's a lot that will always be lazy unless they're made to do it. I'd rather be a Jamadji than a white person, though; Jamadjis get on better. The white person's got to keep everything up to date—the Jamadji is satisfied with what he got; he does not want a lot—he makes that do."

Certain patterns of behavior, including excessive drinking, have become associated with life in native camps; frequent arrests on liquor

charges and terms of imprisonment are the consequence. Often these arrests are due to the natives' habit of settling grievances by informing on one another to the police. This informing occurs among even close relatives. Frequent quarrels and fights are caused by the fear that someone has been spreading gossip or rumors. There is a fear of being talked about and gaining a "bad name." This acts as a social pressure, and helps to enforce the mutual sharing of food and possessions with other natives, even strangers, but more frequently with relatives. One can never refuse to feed another person or give him shelter in one's camp. "If you don't feed them, they talk and give you a bad name." This practice has often been abused, especially by young men who live on their relatives and seldom go to work. During their terms of unemployment they gamble with wage earners and old-age pensioners or with others who have some money, and then spend the proceeds. Generally, however, there is considerable reciprocity, and those who have money share it or use it to gamble with those who have none, only themselves to be supported and fed on some other occasion.

The natives who go "the white way" are those who are prepared to cut themselves adrift from the camp groups and model their behavior on "white" values, as by saving money to buy a house with decent furniture, a refrigerator, electric light, baths, and other modern devices. Camp natives generally own portable radios, beds, and old cars, but their possessions are of a makeshift kind and are allowed to deteriorate. The natives who wish to assimilate are especially concerned about ridding themselves of the stigma of their origin, and avoid all social contacts with the camp natives. They try to associate with white people as much as possible, but their opportunities for social intercourse with whites are often limited by the prejudice that still exists among many country white people against anyone who has aboriginal features.

So long as the white community continues to reject those of aboriginal descent, there is little likelihood that the majority of Jamadjis will have any real incentive to live the "white way." One promising feature of the current situation in Western Australia is the increasing emphasis upon education of the young native children. Some of them have already managed to complete their schooling and engage in certain white-collar occupations. These young people may do much to dispel the idea, still prevalent in the minds of so many Australians, that aborigines are innately inferior to white people. Many natives who have been given social and educational opportunities have shown themselves to be fully as capable as any white citizen.

General References

ANNUAL REPORT. 1956. The Commissioner of Native Welfare. Govt. Printer (1957). Perth, Western Australia.

BELL, J. 1956. The economic life of mixed-blood aborigines on the south coast of New South Wales. Oceania. 26: 181.

BERNDT, R. & C. BERNDT. 1951. From Black to White in South Australia. Cheshire. Melbourne, Australia.

CALLEY, M. 1956. Economic life of mixed-blood communities in northern New South Wales. Oceania. 26: 200.

ELKIN, A. P. 1954. The Australian Aborigines: How to Understand Them. 3rd ed. Angus & Robertson. Sydney, Australia.

ELKIN, A. P. 1951. Reaction and interaction—a food gathering people and European settlement in Australia. Am. Anthropol. 53: 2.

FINK, R. A. 1957. The caste-barrier: an obstacle to the assimilation of part-aborigines in north-west New South Wales. Oceania. 28: 100.

REAY, M. & G. SITLINGTON. 1948. Class and status in a mixed-blood community. (Moree, N. S.W.) Oceania. 18: 179.

REAY, M. 1949. Native thought in rural N. S. W. Oceania. 20: 89.

THE NEW YORK ACADEMY OF SCIENCES

SECTION OF OCEANOGRAPHY AND METEOROLOGY

OBSERVATIONS ON THE UTILITY, THE LIMITATIONS, AND THE DIDACTIC VALUE OF SYNOPTIC STREAMLINE ANALYSIS*

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The Weather Problem, Tropical and Otherwise

This paper describes results of experimental streamline analysis at Miami, which has been gratifying in providing improved understanding of phenomena that have been not only impossible to forecast, but that often are inexplicable in ordinary synoptic terminology even after their appearance.

In natural science it is important to remember that our theories must be fitted to nature, not vice versa. The philosopher A. N. Whitehead, himself a mathematician of standing, warned that "there can be no true physical science which looks first to mathematics for the provision of a conceptual model."[1] It seems likely that repeated, intimate contact with ordinary weather is necessary to the development of useful meteorological theory, and that lack of such contact contributed to the inadequacies of the wave theory of cyclone development, which is now known to describe exceptional rather than ordinary atmospheric behavior. The concept of a front as a zero-order density discontinuity also suffered from inadequate observation: soundings that became available later failed to support the assumed discontinuity, and the theory was revised to call for a first-order discontinuity involving the temperature gradient instead. Even this concept has given ground to an expanding empiricism, and we now recognize that adjacent air masses can and often do merge with no sharp discontinuity of any kind.

We now realize, too, that the classic cold front thunderstorm is a rare event. Even in middle latitudes (Chicago, Ill.) thunderstorms rarely, if ever, occur with cold frontal passage, but typically well ahead of it,[2] and in Florida, where divergence between book weather and real weather is pronounced, the cold front thunderstorm has been called a myth.[3] Misunderstandings arise between forecasters and pilots trained on cross sections showing the thunderhead astride the cold front: too often any line of build-ups, tropical or otherwise, is termed a front. Forecasters frequently must remind pilots that Florida rains are heaviest in summer when the fronts are nonexistent. J. M. Austin and R. H. Blackmer have termed it "impossible" precisely to predict cold front rain with conventional forecast parameters;[4] they note that the long shower bands of

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middle latitudes are not fronts. Plainly, the concept of the cold front thunderstorm needs re-examination.

A Foolish Question

Whitehead also noted that ancient science stopped with Archimedes because men stopped asking foolish questions. The streamline analyst finds himself speculating whether wind may not be as much (or more) a cause of pressure gradients as (than) pressure gradients are a cause of wind. He may note that, at a fundamental level, hydrostatic pressure on unit surface is a function of – indeed is measured only by the intensity of – molecular bombardment, which is tantamount to saying, of molecular motion. After all, wind is merely a large-scale disturbance of mean molecular velocities. A weatherman observing the Bernoullian oscillation of the water level in a water closet during a hurricane may wonder about the value of similarly fluctuating pressure values, responding to the surges of the wind, in calculating this wind. Does the wind blow because the barometer falls, or vice versa? Air movement, being mass molecular movement, derives basic properties from these microlevels; yet the meteorologist traditionally thinks in terms of the motion field as a function of the pressure field, extenuating the power of the motion field to alter the pressure field. V. P. Starr's finding that the classic atmospheric circulation pattern "turned out to exist only in textbooks," with actual large-scale overturning apparently going "the wrong way," suggestive of rising cold air and sinking warm air,[5] seems to this author to call into question much meteorological orthodoxy regarding the role of pressure gradients as a prime mover of the earth's air.

At any rate, pressure analysis is only one of several approaches to weather analysis. Precipitation can be considered a function of the wind field and, if this is analyzed directly, errors are less likely than when it is computed through the confusing intermediary of pressure. We are also less likely to force subjective patterns on nature if we base our analysis directly upon observations rather than on derived, theoretical, and inevitably simplified speculations involving the pressure field.

The Meteorological Asymptote

C. E. Palmer lists, among others, the following empirical facts about low-latitude cloud lines: (1) cyclonic vorticity is not necessary; (2) they may or may not show a trough in the pressure field; (3) they may develop within air that is apparently homogeneous; and (4) they do not move with the wind, nor are pressure changes useful in forecasting the movement. We have, he notes, "placed much emphasis on the 'trough' in the wind field... but... have completely missed the asymptotes of the field which are... accurately located only by using the standard methods described by J. Bjerknes."[6]

The meteorological asymptote is defined as follows: "A streamline in the wind field away from which neighboring streamlines diverge (positive) or toward which they converge (negative)."[7] Presence of a negative asymptote does not always mean weather, of course, for in itself this tool assesses neither diffluence nor speed divergence (isotachs may be added to the analysis for this purpose). Nevertheless, experience has shown that the asymptote is a valuable analytic tool. The negative asymptote sometimes appears to be analogous to a front, manifesting first-order discontinuity in the orthogonal curvature just as a front shows similar discontinuity in the pressure. What is true in a general sense, however, is that air from different sources is brought progressively into such close proximity that the two flows effectively merge into one. Frequently we see that what at first appears to be smooth, homogeneous flow undistinguished by cyclonic vorticity, cyclonic curvature, or speed convergence – an area that would not conventionally come under suspicion as a weather source – is in reality an inconspicuous "front" between air masses of quite different origins.

The synoptic importance of the asymptote may be explained in this way. If *a*, *b*, *c*, *d*, *e*, *f*, and *g* are successive points in a line transverse to the general flow in a confluent field, it is easy to see that *a* and *c*, and *e* and *g*, may approach *b* and *f* asymptotically. However, *b* and *f* in turn approach *d*, which is on what we may term the major asymptote, which is seen as a limit approached by all participating or lesser asymptotes. Its importance stems from the fact that along a single axis may be brought to bear the cumulative confluence of a very large wind field. One difficulty in practical use of the asymptote is determining the *major* asymptote: an area may contain an infinite number of asymptotic streamlines; the forecasting problem consists of deciding which of these will grow at the expense of others. Often data are insufficient to decide this; one wonders if a solution of the problem would not, indeed, require an infinite number of observations. The concept of "participating" and "major" asymptotes derives support from typical radar pictures in which the cloud lines consist of two or three proximate, quasi-parallel lines, straight or curved, one of which will be longer and wider than the others.

There are asymptotes in the vertical sections also, but vertical wind data are not available. We may also note that streamlines do not portray actual paths of air particles, being no more than snapshots of instantaneous conditions.

The negative asymptote differs significantly from the classic front in that nature seems to favor it and is more inclined to use it to produce weather. Whitehead's warning reminds us that we should aim at a minimum of speculation over the properties of the asymptote and at a maximum of observation of them. N. Shaw,[8] too, has urged that meteorology give proper attention to the observational basis of scientific knowledge:

"The true course of progress is to... develop the representation of the motion to such a degree of perfection that the forces will be deduced from it, instead of supposing that we can specify the forces and that nothing but the method of fluxions is necessary to deduce the motion." We may expect, then, to profit less from sophisticated manipulation of the pressure field than from careful observation of the facts of motion. Indeed, such discoveries as the hurricane bands, the jet stream, the inward-spiraling anticyclones, the growth of cumulus clouds through entrainment into their windward sides rather than through a warm, rising dome of air under the cloud, and others have derived entirely through empiricism.

Streamline-isotach analysis is fully described by the Oahu group.[9] With practice, analysis can be greatly speeded through freehand entry of streamlines; it is only necessary to abandon the habit of ignoring (that is, smoothing) reported winds, remembering that any streamline crossing a plotted wind arrow at any angle, or which could not be derived by interpolation from adjoining winds, is an incorrect one. I have prepared a list of *Practical Rules for Rapid Streamline Analysis Without Use of Isogons* that is available to interested persons.

Convergence in one horizontal level cannot relate to weather except, of course, as that level relates to others adjoining it. In practice we may employ a low-level analysis where it can be assumed that convergence means upward motion, together with a higher-level analysis where the effect of superimposed divergence can aid, or restrict, lower thrusts. A favorite lower level in Florida is 7000 feet since, at Miami, showers have been quantitatively related to the 800 millibar mixing ratio.[10] When tropical air covers the area under analysis (a 10° Celsius dewpoint at 850 mb. may be used as a criterion), the negative asymptotes are frequently accompanied by cloud lines, the kind of weather that is mystifying in terms of conventional surface or upper air analysis.

The negative asymptote is not always accompanied, of course, by a cloud line, nor do shower areas necessarily mean presence of an asymptote that is evident on a synoptic scale. Tropical speed surges may be accompanied by shower areas in a field of zero orthogonal curvature. The asymptote is nevertheless worth following. It provides badly needed understanding of the 10-inch tropical rains that sometimes enter south Florida in narrow lines oriented with middle-level winds, and of the east-west lines of cumulonimbus so frequently reported by pilots flying in the Caribbean and showing little relationship to the mysterious "fronts," "troughs," "isallobaric lows," or "waves" drawn on our weather charts. It also provides insight into the origin – indeed, into the necessity – of the spiral bands so conspicuous in tropical lows. There is even, as we shall see, reason to think that the so-called middle-latitude "line squalls," "cold fronts aloft," "instability lines," and "pseudo-

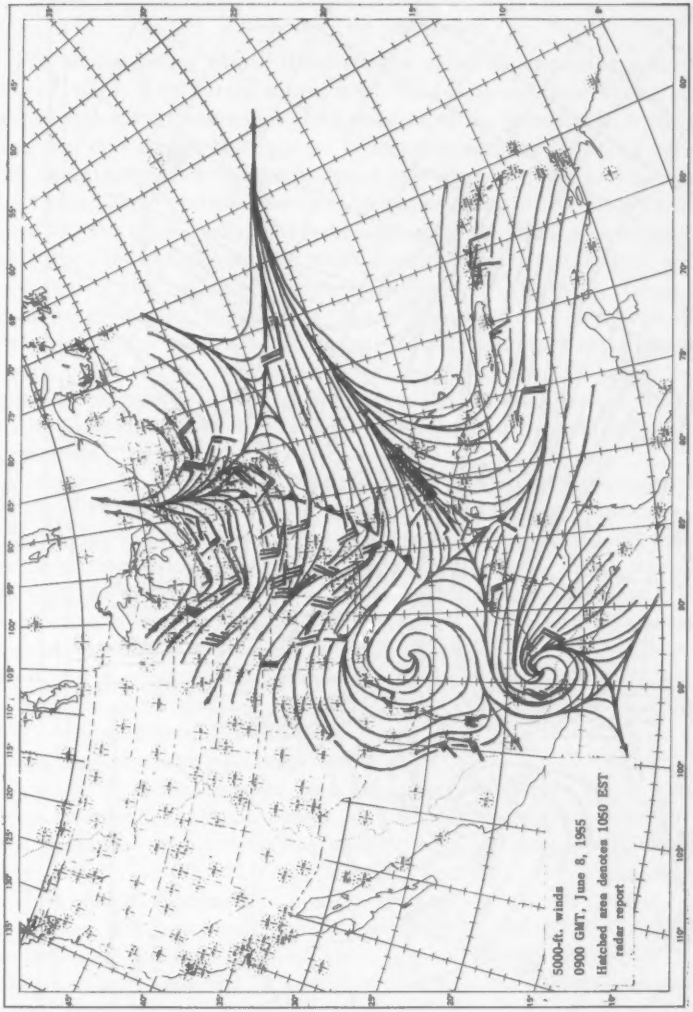
5000-ft. winds
0900 GMT, June 8, 1955
Hatched area denotes 1050 EST
radar report

FIGURE 1

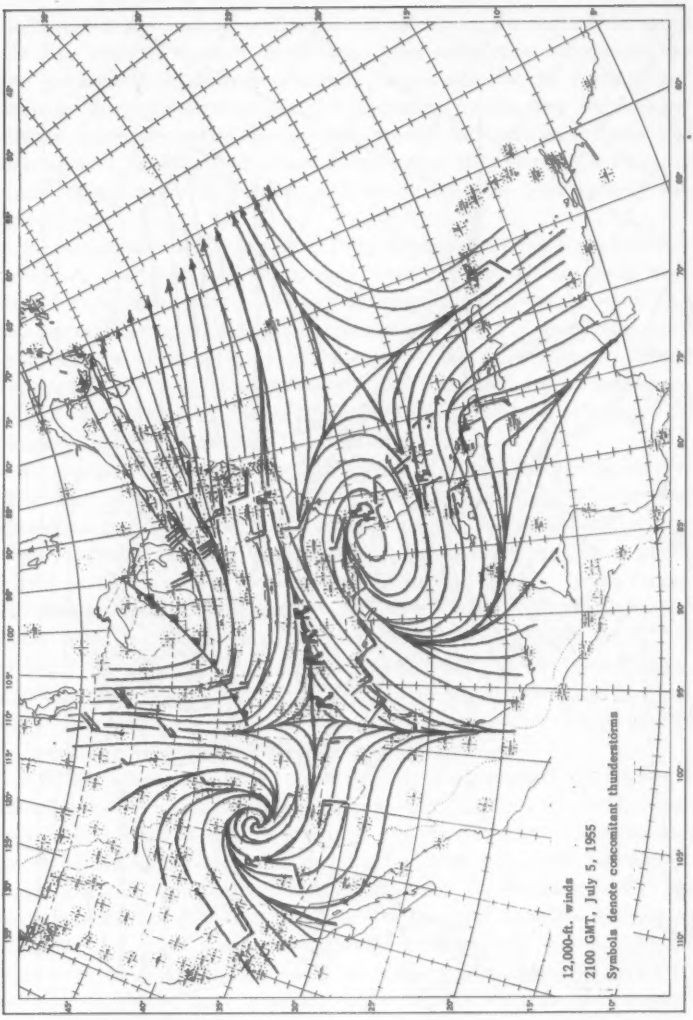
12,000-ft. winds
2100 GMT, July 5, 1955
Symbols denote concomitent thunderstorms

FIGURE 2

cold fronts" (after Bergeron) are in fact negative asymptotes like those drawn by the Oahu group, in which well-marked major asymptotes precede typical cold fronts by 50 to 300 or more miles.

Examples of Analysis

The accompanying charts illustrate the value of streamline analysis. In FIGURE 1, a cold front across north Florida is preceded by a typical belt of active thunderstorms well in advance of the front itself. It will be seen that this shower belt appears to coincide with the 0900 GMT asymptote emerging from the hyperbolic point just west of Cuba. In FIGURE 2 the "squall line" precedes the front by considerably more than 300 miles, provoking the impatience of the forecaster using the front as an analytical tool. Even the thunderstorm in North Carolina can be interpreted as an extension of the negative asymptote running from Ft. Worth, Texas, to Chattanooga, Tenn., and another thunderstorm in the Florida Keys also seems related to a 12,000-foot negative asymptote. Additional thunderstorms in north Florida and northern New Mexico seem to result from negative asymptotes spiraling into anticyclones. It will be noted that nearly all winds surrounding the anticyclonic vortices have strong inward components.

FIGURES 3, 4, 5, and 7 illustrate continuity problems through consecutive 6-hourly observations (FIGURES 6 and 6a are synoptic with FIGURE 5). FIGURE 3 is typical of the complexity often found in the low-level circulation: intricate whorls and eddies are evident, and one suspects that individual charts are quite as unlike as fingerprints.* The predilection of the showers and thunderstorms for the negative asymptotes in this 7000-foot section is typical and noteworthy: one wonders if exceptions may not be due to errors in analysis or in choice of level. Experience shows that patterns often differ radically within relatively short vertical differences, and the problem of deciding which levels are of most significance needs much further study.

FIGURE 4 shows changes that are almost incredible for a 6-hour period. Incredible, that is, except to a forecaster who has noted over many years that weather patterns seldom progress in an orderly fashion across the map as they do in books and in the popular mind but, instead, manifest erratic changes that are, as often as not, abrupt, chaotic, and unexpected. Who, we wonder, would have forecast the 1000 GMT 20-knot northerly wind at Albuquerque, N. Mex., from the flow in FIGURE 3? The cusp point at 23 north and 72 west (FIGURE 4) is the precursor of a new tropical storm. We note again that thunderstorms have a curious predilection for negative asymptotes (while shower distribution seems to be more random). In FIGURE 5 we observe numerous and new radical changes;

*Inspection of one's own fingertips or hair patterns will reveal that singular points and asymptotes are no strangers to nature.

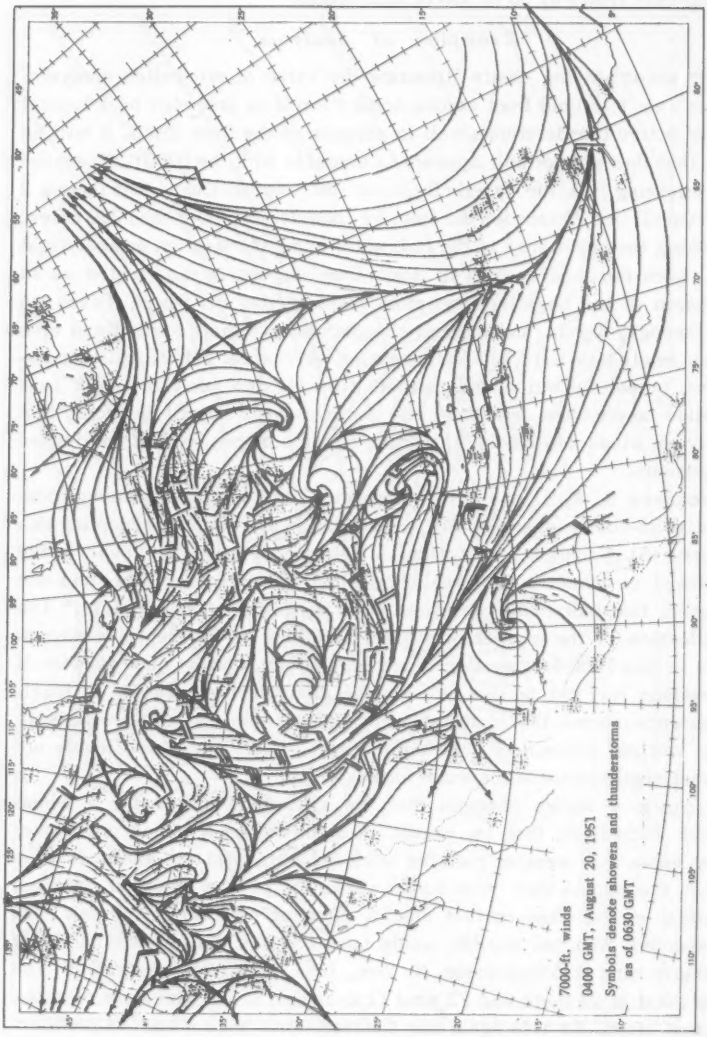
7000-ft. winds
0400 GMT, August 20, 1951
Symbols denote showers and thunderstorms
as of 0630 GMT

FIGURE 3

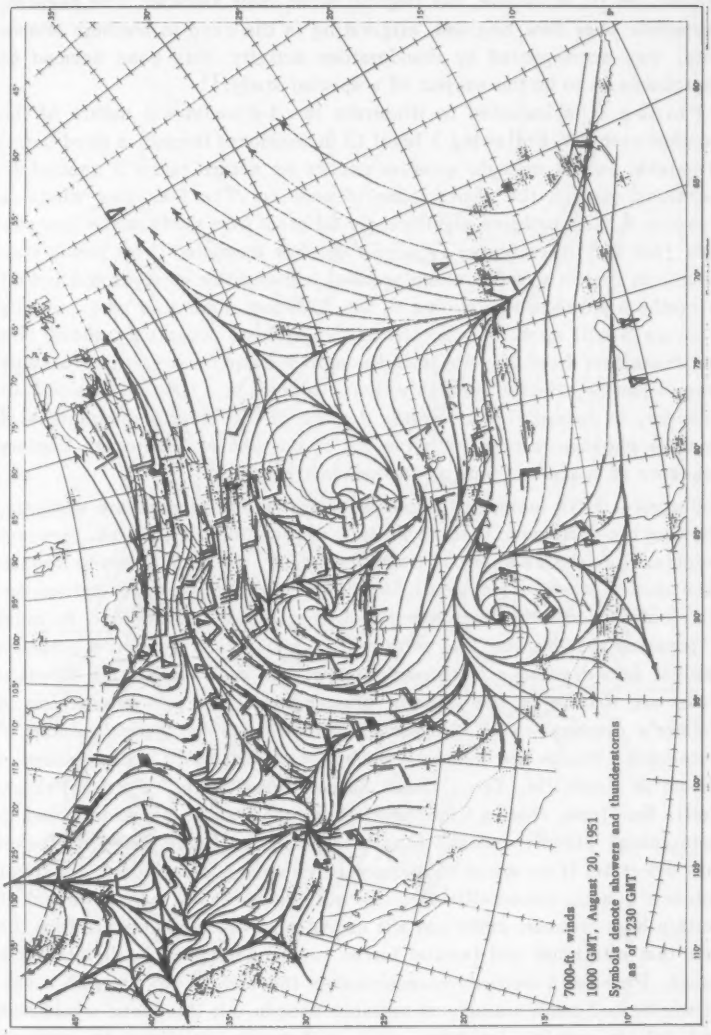
7000-ft. winds
1000 GMT, August 20, 1951
Symbols denote showers and thunderstorms
as of 1230 GMT

FIGURE 4

however, certain features such as the hyperbolic point in the Atlantic, which is continuing its southwestern movement, seem reasonably persistent, as is, of course, the tropical storm over Yucatan. The negative asymptote over New England, originating in the cusp in northern Minnesota, was accompanied by thunderstorm activity: this case seemed so improbable as to be the subject of a special study.[11]

FIGURE 6 is included to illustrate the 4-dimensional nature of the weather problem. Following 1 level (2 dimensions) through a third (time) dimension is not enough: weather occurs as nature takes 3 spacial dimensions through the fourth (time) dimension. The 5000-foot winds in FIGURE 6 show patterns significantly different from those in the synoptic data just 2000 feet higher (FIGURE 5). For example, if he seeks continuity in the form of the singular point marking the anticyclonic indraft in northern Montana in FIGURE 6, the 7000-foot specialist who has only FIGURE 5 will seek in vain. Streamline analysis repeatedly shows that the transition from smooth flow through a cusp to a vortex – or vice versa – can be effected through either a spacial or a time dimension: one wonders, in speculative moments, if some of the incomprehensibility of weather phenomena may not arise out of our use of Nineteenth Century concepts of space and time as independent realities!

FIGURE 6a is included for comparison of standard pressure analysis, showing the same wind data as FIGURE 6, together with 850 mb. pressure heights. Note the weatherless trough or "front" from Minnesota to Kansas (see shower areas in FIGURE 6). Seemingly, it is convergence and asymptotic confluence rather than shear that is productive of weather. At least 2 pressure heights on the northwestern gulf coast lead the pressure analyst into drawing a fictitious anticyclone: such misleading observations are routine in low-latitude pressure analysis and remind one of Palmer's discussions of "intolerable uncertainty" in these latitudes.[12] Heterodox – though not unusual – movement of air toward high pressure is shown at Nashville, Tenn., Sault Sainte Marie, Mich., Port-au-Prince, Haiti, San Juan, Puerto Rico, and other areas where air unschooled in meteorology blandly crosses height isopleths at fairly sharp angles in this direction. If we are to base our science upon observation (and natural science can be based ultimately on nothing else) we must here ask if certain high-pressure cells may not derive most of their pressure from the fact that rotational and inertial forces are funneling much air into little space. Perhaps a *Gedankenexperiment* is in order: if we imagine a uniformly heated earth rotating at variable speeds, air thickness would rise and fall in varying latitudes, with systematic variations in pressure readings on the earth's surface. Would the earth-bound meteorologist then persist in his faith that such pressure forces and gradients were the cause rather than the effect of air movements?

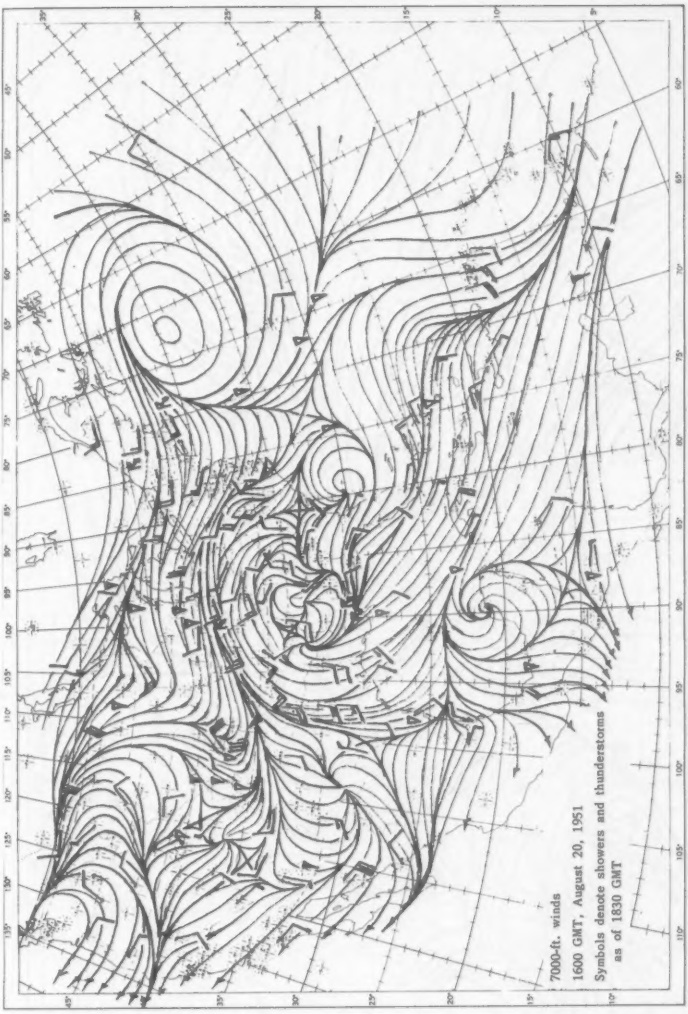
7000-ft. winds
1600 GMT, August 20, 1951
Symbols denote showers and thunderstorms
as of 1830 GMT

FIGURE 5

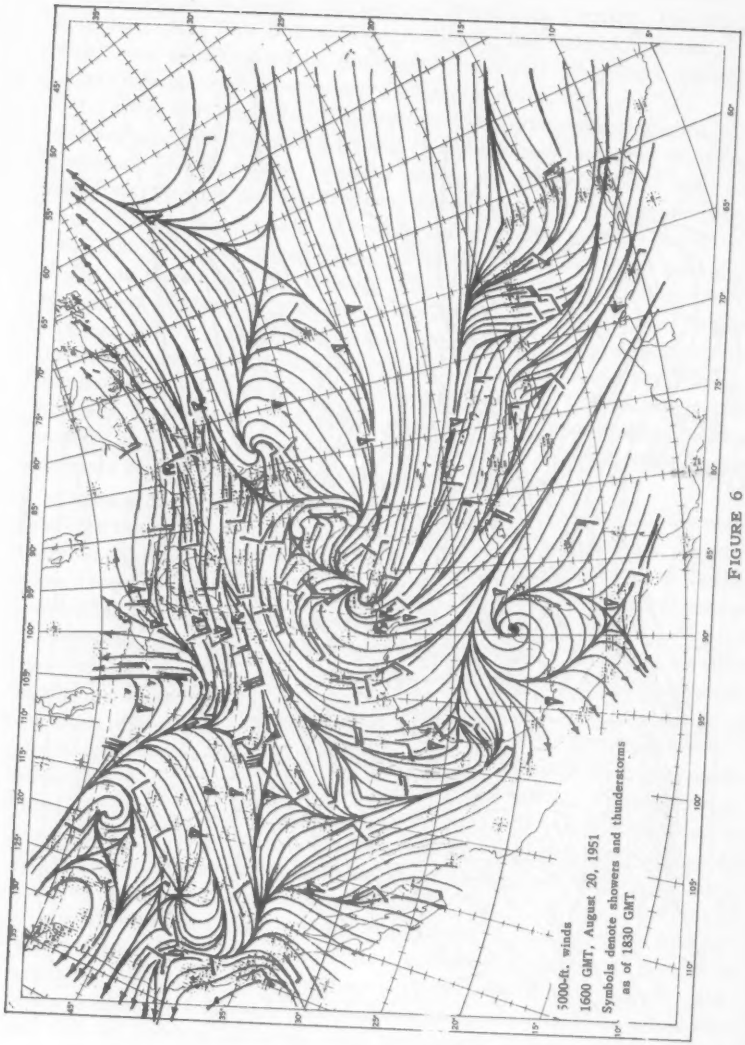
5000-ft. winds
1600 GMT, August 20, 1951
Symbols denote showers and thunderstorms
as of 1830 GMT

FIGURE 6

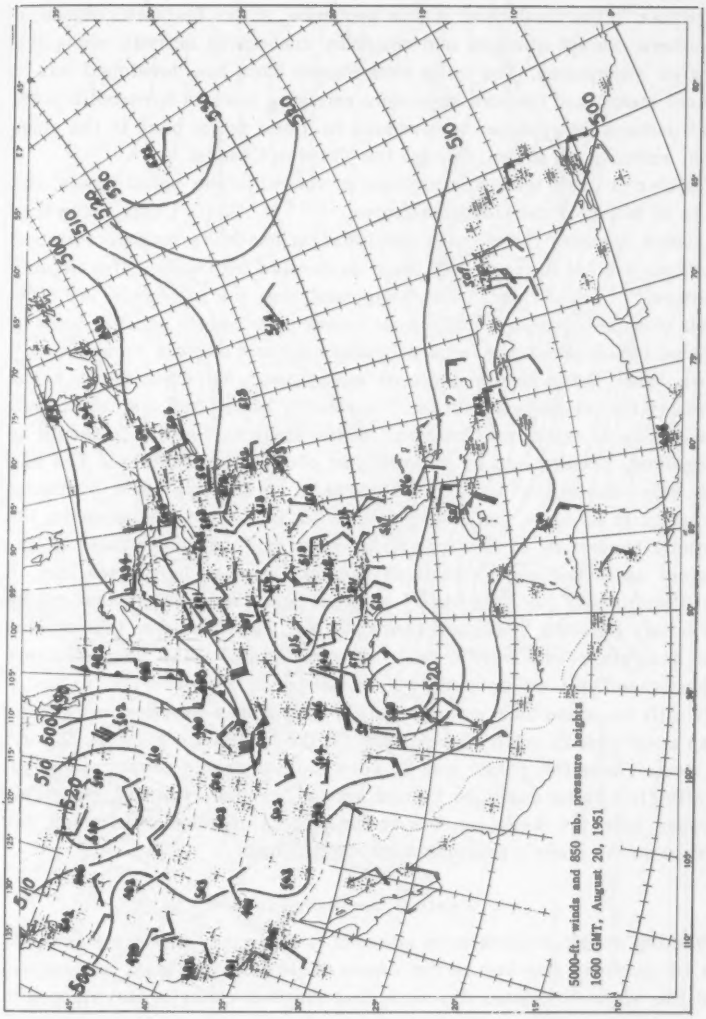
5000-ft. winds and 850 mb. pressure heights
1600 GMT, August 20, 1951

FIGURE 6a.

Perhaps the most striking aspect of the comparison of FIGURES 6 and 6a is the superior portrayal of the microstructure by streamline analysis. It is this microstructure that is related to actual weather patterns.

FIGURE 7, the final chart of this sequence, shows features common to the others: abrupt changes and maximum convective activity along the negative asymptotes. The cusp near Puerto Rico has developed into a tropical storm, and the new asymptote emerging from its hyperbolic point could perhaps be expected to give rise to a new spiral band in the older storm, entering, as shown, through the Yucatan Channel.

FIGURE 8 deals with this problem of the hurricane spiral bands, the cause of which is considered unknown.[13-14] M. G. H. Ligda notes that "in some respects these outer precipitation bands have characteristics which are similar to instability lines associated with some extra-tropical cyclones."[13] H. Wexler[15] has suggested that the rainbands are "the result of superimposing a convergent vortex onto certain parallel lines of cumulus clouds which are frequently observed over tropical ocean areas." If we label these bands negative asymptotes, we establish a sound homology for comparison with the "instability lines" and also show their relationship to the cloud "streets" of the tropics. The bands should be recognized, however, as an integral part of the hurricane itself. The fact that only streamline analysis is capable of showing their kinematic necessity in any low, tropical or otherwise, is a strong argument for the intrinsic superiority of this type of analysis: no longer need there be any mystery as to the huge, comma-shaped rain areas of hurricanes, nor to the "prefrontal" or "air-mass" showers and shower lines that nature stubbornly persists in manufacturing. In FIGURE 8 we see that streamline analysis seems fully capable of accounting for the band structure of hurricane Dolly as reported by R. H. Simpson.[16]

It will be noted that major negative asymptotes emerge from (1) the cusp point near 20 north 70 west, and (2) the hyperbolic point at 22 north 76 west. These two points may be keys to this storm's supply of energy; if effective gates could be closed across (or chain-reacting chemicals dropped into) the weak currents at the origins of these asymptotes, the effects on the storm's progress might be profound.

Is Weather Indeterminate?

Nothing in this discussion is intended to imply that streamline analysis per se provides any key to the *cause* of weather. We must not assume that the windmill creates the wind, that the ever-changing asymptotes in the surface soap film make the water go down the drain. Asymptotes are closely related to current and short-term weather, but within my knowledge and experience their forecasting is essentially an unsolved problem.

Recalling the experiences of the alchemists, who were less concerned with the *if* than the *how* of obtaining gold from lead, one wonders if

7000-ft. winds
2200 GMT, August 20, 1951
Symbols denote showers and thunderstorms
as of 0030 GMT, August 21, 1951

FIGURE 7

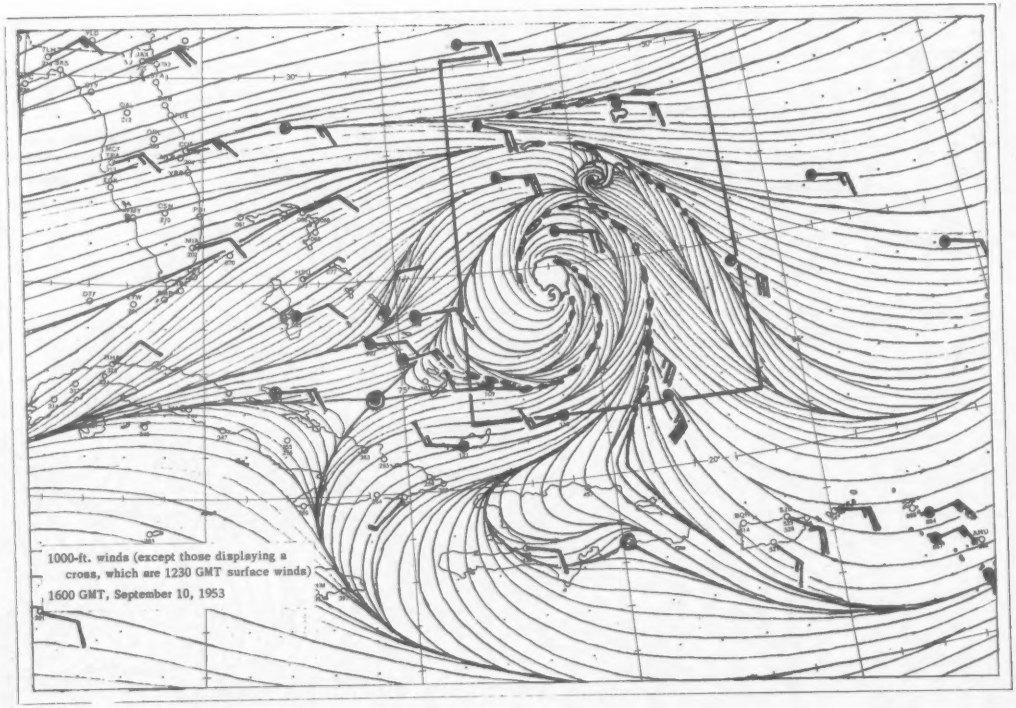

FIGURE 8. Note that the solid line encloses a reproduction of the cloud microstructure at 1500 GMT of this hurricane (Dolly) as reported by Simpson.[16]

meteorologists have spent enough time asking if our atmosphere is really a determinate physical mass at all. It is commonly assumed – on faith – that future weather is predetermined by present conditions. Rainmaking activity, if it does little else, should shake our faith in this belief. Nor has experience with streamline analysis arrested my gradual development of a belief in the basic indeterminacy of all weather phenomena. The meteorologist is and will remain ignorant of even such basic information as the freezing point of atmospheric water, for the exact composition of the air is both unknown and subject to unforeseeable changes: every automobile exhaust, every industrial smokestack, every volcanic eruption, every forest fire, every breaking ocean wave, every meteoritic dust shower ensures the fact that the meteorologist, unlike the physicist and the chemist, can never solve his problems with certainty.

With important branches of physics established as fully indeterminate, it is paradoxical that meteorology – almost a popular synonym for uncertainty – has been diffident toward the idea that in weather, too, there are fundamentally indeterminate situations. An overwhelming if taciturn body of forecasting experience indicates that this is true. The weather forecaster, unlike Einstein, finds it easy to believe that "God plays dice with the cosmos." The suggestion has arisen, never being taken very seriously, that weather is effectively, and perhaps fundamentally, indeterminate;[3] another author believes that an adequate knowledge of atmospheric processes may lead only to a better understanding of the "inherent unpredictability of the weather."[17] Herein may lie the meaning of an unfortunate but distinct tendency for meteorologists with excellent formal training to remove themselves from situations requiring personal issuance of routine forecasts. Adoption of the concept of indeterminacy might ameliorate many ills of meteorology: the anxiety present in the forecaster-stereotype of the comic page suggests that any security based on an incorrect understanding of nature cannot in the long run prove satisfactory and lasting. Such respected and rewarding fields as law and medicine accept and cope daily with natural indeterminacy. Unlike meteorologists, however, these professionals have never yielded to popular demands that uncertainty be converted to certainty; forecasts are issued only if and when conditions warrant. Though cases may be lost and patients may die, dignity is maintained by distinguishing limited human abilities from natural uncertainty, for which no apology is needed.

In the famous Michelson-Morley "ether drift" experiments, physicists were faced with two alternatives: either experiments had been carelessly conducted, or all concepts based on absolute space were fallacious. Einstein accepted this bitter pill because it provided the only solution. The phrase "conspiracy of nature" had come into use as physicists gave vent to a feeling that nature was hiding her ways. This "conspiracy" was eventually recognized as an undiscovered law of nature, one that

replaced human concepts of space and time with the space-time continuum.

Meteorology seems to face a similar conspiracy. We know that the general equations of hydrodynamics are nonlinear partial differential equations; that methods for solving any but very special kinds do not exist. Substitute equations, dictated by mathematical convenience rather than observation of nature, violate Whitehead's maxim. The possibility looms that our failure to obtain practical solutions is nature's way of saying that these do not exist, that weather is in fact indeterminate—probabilistic rather than deterministic. Some assured forecasts can be made, of course, but such situations do not occur every hour on the hour for the convenience of the public, and even these seem to be cases of high probability rather than certainty. Cases doubtless exist in which the probability is exactly one half. Instead of being prodded to reduce hedging, which is often simple honesty analogous to the doctor's "I don't know," forecasters should be reminded that to speak with assurance under doubtful conditions is both unwarranted and dishonest. I believe that strict adherence to this principle could not only reduce public distrust of the weatherman, but would improve his own self-esteem. A deterministic, exact solution to the weather problem may yet turn out to be, like the ether, mere anthropomorphic bias stemming from human needs and limitations.

Merits and Limitations of Streamline Analysis

The chief value of streamline analysis seems to be that it accounts for weather patterns that are otherwise inexplicable. Another advantage is that placement of asymptotes and singular points, within a given network, is far less arbitrary than is the ordinary placement of fronts. Flow patterns are obvious to all when we abandon preconceived ideas about what winds *should* do and accept, instead, what they *do:* meteorological analyses require "coordination" only when we subvert natural patterns in favor of those in our heads. Streamline analysis is grounded not in speculation but in observation.

An important limitation of streamline analysis appears to be poor time continuity. A. G. Forsdyke observed that the equatorial "front" also appears to jump from point to point without passing through intermediate points.[18] Such apparent behavior may result from time intervals that produce effects such as backward-moving spokes seen in motion pictures; it may also, as has been noted, be due to lack of four-dimensional analysis. If pressure analysis seems to offer better continuity, we note that what is desperately needed is not continuity in itself, but an analysis that fits observed weather closely. We must follow where the trail leads. R. W. Pinder has suggested, in discussions of this problem with me, that poor continuity is really an encouraging sign since weather, too, manifests poor continuity both in space and time.

It may be objected that, since streamline analysis is related chiefly to short-term weather, it is of little value in forecasting for normal periods. This view provokes thought, and leads one to ask in reply how meteorology can hope to run before it can walk.

A problem arises out of the ease with which asymptotes can be shifted hundreds of miles by relatively slight shifts (or errors) in reported winds. Some meteorologists feel that pibal accuracy, at least by the time observations are plotted for use, is not great enough to locate asymptotes with the required exactness. To a large extent the ephemeral, momentary character of the streamlines accurately reflects the unstable, rapidly varying condition of the atmosphere itself. It should, however, be pointed out that repeated experience has shown that ordinary plotted winds have located cloud lines that could be accounted for in no other way. A forecaster who has once experienced this thrill—after a decade or two of groping in the dark—will worry less about representativeness of pibals than about his own selection of levels for analysis. He may also point out that if he cannot believe the observations he has, he should discontinue his endeavors. If it is to be objective, his analysis of nature must be based on available observations and on nothing else.

Low-latitude forecasters have rarely been able to produce accurate forecasts for long periods, but several short-period (1- to 12-hour) clues to south Florida summer shower probabilities are known.[10] These include, among others, a low Showalter index; 850 mb. dewpoints of over 10° C., particularly with isentropic uplift; convective instability in low and middle levels and generalized vertical motions that release this, as at Miami where such diurnal motions have been established;[19] large 72-hour falls in the 700-mb. height; and lowering of the freezing level. To some extent all these criteria overlap and are no more than different ways of saying the same thing. It is worthy of note that many of these tropical shower parameters are found regularly along the low- and middle-level negative asymptotes, which are easier to follow than are the separate items.

If streamline patterns provide information that is of short-range value only, the fact remains that even this is often otherwise unavailable. Also, the problem of indeterminacy suggests that we should bring into sharper focus those aspects of forecasting that are most reliable, for it is in the short-term trends that indeterminacy would be easiest to detect and to understand.

The broadest potentialities of streamline analysis, however, may lie beyond any mere technical aids inherent in the analysis itself. I have been astonished suddenly to see plainly and, in a small way, to understand phenomena that have escaped my understanding for many years. However, these manifestations can be seen only by adopting the meteorologically unsophisticated procedure of accepting basic observations

at face value and of allowing the wind to tell its own uninhibited story. Louis Agassiz's admonition to "study nature, not books" is, one suspects, no less applicable to weather science than to other natural science. Perhaps it will take root and grow in meteorology.

Summary

Evidence is presented suggesting that ordinary low-latitude shower lines, typically unrelated to fronts, cyclonic vorticity, or pressure troughs, are closely related to the negative asymptote. It is further suggested that this relationship may provide a basis for establishing physical homologies in such diverse phenomena as the middle-latitude squall lines and the hurricane spiral bands, with thunderstorms occurring principally along the negative asymptote.

The utility of streamline analysis in forecasting appears to be limited by poor time continuity, a feature further suggesting a relationship to severe weather phenomena. Experience with streamline analysis leads to a concept of weather as a four-dimensional phenomenon difficult to portray and understand in two- or three-dimensional media. The trail of empiricism also leads to anticyclonic inward-spiraling and to the problem of indeterminacy.

Acknowledgment

I am indebted to Wilmer L. Thompson, E. J. Christie, and J. W. Osmun for their encouragement and assistance. I am also grateful to Gordon E. Dunn for providing a short course in streamline analysis at Miami, Fla., in 1955, which was conducted by Noel LaSeur and the late Leon Sherman.

References

1. WHITEHEAD, A. N. 1922. The Principle of Relativity, with Applications to Physical Science. : 39. Cambridge Univ. Press. Cambridge, England.
2. MEANS, L. L. 1952. On thunderstorm forecasting in the central United States. Monthly Weather Rev. **80**: 172.
3. CARSON, R. B. 1954. On the nature of the weather problem. 133rd Natl. Meeting Am. Meteorol. Soc. Miami Beach, Fla.
4. AUSTIN, J. M. & R. H. BLACKMER, JR. 1956. The variability of cold front precipitation. Bull. Am. Meteorol. Soc. **37**: 453.
5. STARR, V. P. 1956. The general circulation of the atmosphere. Sci. American. **195**(6): 40-45.
6. PALMER, C. E. 1951. Tropical meteorology. *In* Compendium of Meteorology. : 874-875. Am. Meteorol. Soc. Boston, Mass.
7. PALMER, C. E., C. W. WISE, L. J. STEMPSON & G. H. DUNCAN. 1955. The Practical Aspect of Tropical Meteorology. : 68. Oahu Research Center. Inst. Geophys. Univ. Calif.
8. SHAW, N. 1942. Manual of Meteorology. **1**: 322. Cambridge Univ. Press. Cambridge, England.
9. PALMER, C. E., C. W. WISE, L. J. STEMPSON & G. H. DUNCAN. 1955. The Practical Aspect of Tropical Meteorology. Oahu Research Center. Inst. Geophys. Univ. Calif.

10. CARSON, R. B. 1954. Some objective quantitative criteria for summer showers at Miami, Florida. Monthly Weather Rev. 82: 9-28.
11. PENN, S. 1955. A possible high level thunderstorm mechanism in the case of August 20, 1951, over New England. Bull. Am. Meteorol. Soc. 36: 278-288.
12. PALMER, C. E., C. W. WISE, L. J. STEMPSON & G. H. DUNCAN. 1955. The Practical Aspect of Tropical Meteorology. : 1. Oahu Research Center. Inst. Geophys. Univ. Calif.
13. LIGDA, M. G. H. 1955. Hurricane squall lines. Bull. Am. Meteorol. Soc. 36: 341.
14. SIMPSON, R. H. 1954. Hurricanes. Sci. American. 190(6): 34.
15. WEXLER, H. 1947. Structure of hurricanes as determined by radar. Ann. N. Y. Acad. Sci. 48(8): 836-837.
16. SIMPSON, R. H. 1954. Structure of an immature hurricane. Bull. Am. Meteorol. Soc. 35: 339.
17. GRINGORTEN, I. J. 1955. Methods of objective weather forecasting. Advances in Geophys. 2: 84.
18. FORSDYKE, A. G. 1949. Weather forecasting in tropical regions. Geophysics Mem. No. 82.
19. DAY, S. 1953. Horizontal convergence and the occurrence of summer precipitation at Miami, Florida. Monthly Weather Rev. 81: 155-161.

CONFERENCES HELD

SECTION OF BIOLOGY

AXENIC CULTURE OF INVERTEBRATE METAZOA: A GOAL

Monday, April 7, and
Tuesday, April 8, 1958

Conference Chairman: Ellsworth C. Dougherty

Department of Physiology, School of Medicine, and Laboratory of Comparative Physiology and Morphology of the Kaiser Foundation, University of California, Berkeley, Calif.

MONDAY, APRIL 7, 1958

Session Chairman: Seymour H. Hutner

Haskins Laboratories, New York, N. Y.

9:00 A.M. –

Greetings from the Academy – Albert S. Gordon, Chairman, Section of Biology, The New York Academy of Sciences, New York, N. Y.; New York University, New York, N. Y.

"Coelenterates and Tissue Symbionts" – John J. A. McLaughlin and Paul A. Zahl, Haskins Laboratories, New York, N. Y., and St. Francis College, Brooklyn, N. Y.

"pCO_2 Control of Sexual Differentiation in *Hydra*" – W. Farnsworth Loomis, Loomis Laboratory, Greenwich, Conn.

"Preliminary Studies on the Axenic Cultivation of a Planarian *(Dugesia)*" – Charles A. Miller and Willis H. Johnson, Department of Biology, Wabash College, Crawfordsville, Ind.

"Rotifers as Biological Tools" – Helene A. Nathan and Aimlee D. Laderman, Haskins Laboratories, New York, N. Y.; Ellsworth C. Dougherty, Department of Physiology, School of Medicine, and Laboratory of Comparative Physiology and Morphology of the Kaiser Foundation; and Ole Barré, Laboratory of Comparative Physiology and Morphology of the Kaiser Foundation, University of California, Berkeley, Calif.

"Rearing of Certain Nemerteans (genus *Lineus*)" – Marie Gontcharoff, Station Marine d'Endoume, Faculté des Sciences, Université de Marseille, Marseilles, France.

Session Chairman: Ernest Baldwin

Department of Biochemistry, University College, University of London, London, England

2:00 P.M. –

"Maturation of Larval Pseudophyllidean Cestodes and Strigeid Trematodes under Axenic Conditions: the Significance of Nutritional Levels in Platyhelminth Development"–J. D. Smyth, Department of Experimental Biology, Trinity College, University of Dublin, Dublin, Eire.

"Conditions Favoring the Axenic Culture of *Neoaplectana glaseri*, a Nematode Parasite of Certain Insect Grubs"–Norman R. Stoll, The Rockefeller Institute for Medical Research, New York, N. Y.

"Development *in Vitro* of Some Parasitic Nematodes of Vertebrates"–Paul P. Weinstein and Myrna F. Jones, Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases, Public Health Service, Bethesda, Md.

"Axenic Cultivation of the Nematode *Caenorhabditis briggsae*"–Eder Lindsay Hansen, Laboratory of Comparative Physiology and Morphology of the Kaiser Foundation, University of California, Berkeley, Calif.; Warwick L. Nicholas, Department of Zoology, University of Liverpool, Liverpool, England.

"Some of the Problems and Goals of the Axenic Culturing of Nematodes and Other Lower Invertebrates"–Warwick L. Nicholas, Department of Zoology, University of Liverpool, Liverpool, England; Ellsworth C. Dougherty, Department of Physiology, School of Medicine, and Laboratory of Comparative Physiology and Morphology of the Kaiser Foundation, University of California, Berkeley, Calif.

TUESDAY, APRIL 8, 1958

Session Chairman: Ross F. Nigrelli

New York Zoological Society, Zoological Park, The Aquarium, New York, N. Y.

9:00 A.M. –

"Growth of the Schistosome Vector Snail *Australorbis glabratus* under Axenic Conditions"–Eli Chernin, Department of Tropical Public Health, School of Public Health, Harvard University, Boston, Mass.

"Feeding and Nutrition in the Shipworm *(Teredo)*" – Charles E. Lane, The Marine Laboratory, University of Miami, Coral Gables, Fla.

"Monoxenic Culture of a Marine Harpacticoid Copepod"–L. Provasoli, Haskins Laboratories, New York, N. Y.; K. Shiraishi, Tohoku University, Sendai, Japan; and James R. Lance, Scripps Institution of Oceanography, University of California, La Jolla, Calif.

"Studies on a Synthetic Diet for the Scorpion *Centruroides sculpturatus*" – Gordon L. Bender, Department of Biological Sciences, Arizona State College, Tempe, Ariz.

"A Historical and Comparative Survey of the Dietary Requirements of Insects" – G. S. Fraenkel, Department of Physiology, University of Illinois, Urbana, Ill.

"Prolonged Survival and Growth of Insect Ovarian Tissue under *in Vitro* Conditions" – Thomas D. C. Grace, The Rockefeller Institute for Medical Research, New York, N. Y.

"Axenic Culture of Wax Moths for Digestion Studies" – D. F. Waterhouse, Division of Entomology, Commonwealth Scientific and Industrial Research Organization, Canberra, Australia (to be read by Thomas D. C. Grace).

Session Chairman: William Trager
The Rockefeller Institute for Medical Research, New York, N. Y.

2:00 P.M. –

"Minimal Nutritional Requirements of the German Roach *Blattella Germanica*" – Harold T. Gordon, Department of Entomology and Parasitology, University of California, Berkeley, Calif.

"Circumstances Affecting the Quantitative Nutritional Requirements of *Drosophila*" – James H. Sang, Poultry Research Centre, Agricultural Research Council, Edinburgh, Scotland.

"Miscellaneous Nutritional Variations, Environmental and Genetic, in *Drosophila*" – Taylor Hinton, Department of Zoology, University of California, Los Angeles, Calif.

"Nutritional Studies on the Blowfly *Phormia regina*" – Vernon H. Cheldelin and R. W. Newburgh, Science Research Institute, Oregon State College, Corvallis, Oreg.

"Nutrition of Onion Maggots – Larvae of *Hylemya antiqua* – With Reference to Other Members of the Genus *Hylemya*" – W. G. Friend, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Ont., Canada.

"Nutrition of Parasitoid Dipterans of the Genus *Pseudosarcophaga* and of Other Insects" – H. L. House, Entomology Laboratory, Science Service, Department of Agriculture, Belleville, Ont., Canada.

SECTION OF PHYSICS AND CHEMISTRY

THE ANALYSIS OF MIXTURES OF VOLATILE SUBSTANCES

Thursday, April 10 and
Friday, April 11, 1958

Conference Chairman: Emil F. Williams
American Cyanamid Company, Stamford, Conn.

THURSDAY, APRIL 10, 1958

THEORETICAL CONSIDERATIONS

Session Chairman: Paul H. Emmett
Johns Hopkins University, Baltimore, Md.

9:00 A.M. –

Greetings from the Academy – Joseph Greenspan, Chairman, Section of Physics and Chemistry, The New York Academy of Sciences, New York, N. Y.; Process and Instruments, Brooklyn, N. Y.

"Theory of Gas Chromatography Applied to Highly Reactive Short-Lived Reactor Gases" – E. Glueckauf, United Kingdom Atomic Energy Authority, Harwell, England.

"Theory of Choice of Liquid Substrates for Gas-Liquid Chromatography" –J. H. Purnell, Cambridge University, Cambridge, England.

"Theory of Gas-Solid Chromatography" – Jaroslav Janak, Academy of Sciences, Prague, Czechoslovakia.

ROUND TABLE DISCUSSION

Marcel Golay, The Perkin-Elmer Corporation, Norwalk, Conn.
E. Glueckauf, United Kingdom Atomic Energy Authority, Harwell, England.

MEDICINE, PUBLIC HEALTH, AND FOOD CHEMISTRY

Session Chairman: William Insull
The Rockefeller Institute for Medical Research
New York, N. Y.

2:00 P.M. –

"Gas Chromatographic Analysis of Higher Fatty Acids and Fatty Acid Methyl Esters" – R. K. Beerthuis, G. Dijkstra, J. G. Keppler, and J. H. Recourt, Unilever Research Laboratory, Vlaardingen, The Netherlands.

"Plasma Cholesterol Fatty Acids in Human Subjects as Determined by Gas Chromatography and Other Methods" – G. F. Michaels, P. Wheeler, G. Fukayama, and L. W. Kinsell, Institute of Metabolic Research, Highland Alameda County Hospital, Calif.

"Gas Chromatography in Amino Acid Analysis" – M. Bier, Fordham University, New York, N. Y.

"Recent Advances in the Separation of Methyl Esters of Fatty Acids by Gas Chromatography" – C. H. Orr and J. E. Callen, Procter & Gamble, Cincinnati, Ohio.

"Evaluation of Stationary Phases for Fatty Acid Analysis" – Seymour R. Lipsky, Yale University, New Haven, Conn.

"A Multistage Analyzer for Exhaust Gas Analysis" – R. W. Hurn, J. O. Chase, and K. J. Hughes, Petroleum Experiment Station, Bureau of Mines, United States Department of the Interior, Bartlesville, Okla.

FRIDAY, APRIL 11, 1958

GENERAL LABORATORY

Session Chairman: D. H. Lichtenfels
Gulf Research and Development Laboratories, Pittsburgh, Pa.

9:00 A.M.

"Katharometer Behavior" – W. A. Wiseman, Gilbey, London, England.

"The Isomerization of 1-Bromo-1-Propene" – Lewis F. Hatch, University of Texas, Austin, Texas.

"The Gas Chromatographic Analysis of Mixtures Containing Oxygen, Nitrogen, and Carbon Dioxide" – N. Brenner and E. Cieplinski, The Perkin-Elmer Corporation, Norwalk, Conn.

"A Radio Frequency Excited Detector for Gas Chromatography" – Arthur Karmen and Robert Bowman, Laboratory of Technical Development, National Heart Institute, Public Health Service, Bethesda, Md.

"A New Type of Ionization Detector for Gas Chromatography" – J. Lovelock and A. T. James, National Institute of Health, Mill Hill, London, England.

"Properties of Calcium Zeolite as Adsorbent for Gas Chromatography" – Jaroslav Janak, Academy of Sciences, Prague, Czechoslovakia.

PROCESS CONTROL AND INSTRUMENTATION

Session Chairman: B. F. Dudenbostel

Esso Research and Engineering Co., Linden, N. J.

2:00 P.M. –

"Applications of Process-Control Gas Chromatography in the Chemical Industry" – R. F. Wall, W. J. Baker, T. L. Zinn, and J. F. Combs, Monsanto Chemical Company, Texas City, Texas.

"Heatless Fractionation" – C. W. Skarstrom, Esso Research and Engineering Company, Linden, N. J.

"T. C. Detectors for Process Control" – Dean Eanes, Leeds and Northrup, Philadelphia, Pa.

"Adapting Gas Chromatography to Automatic Process Stream Analysis" – H. N. Claudy, C. C. Helms, P. R. Scholly, and D. R. Bresky, The Perkin-Elmer Corporation, Norwalk, Conn.

SECTION OF BIOLOGY

THE INFLUENCE OF HORMONES ON LIPID METABOLISM IN RELATION TO ARTERIOSCLEROSIS

Friday, April 11, and
Saturday, April 12, 1958

Conference Co-Chairmen:

Abraham Dury
Dorn Laboratory for Medical Research, Bradford Hospital, Bradford, Pa.

Carleton R. Treadwell
The George Washington University School of Medicine, Washington, D. C.

FRIDAY, APRIL 11, 1958

METABOLISM OF LIPIDS – BIOSYNTHESIS, ABSORPTION, TRANSPORT

Session Chairman: Forrest E. Kendall
College of Physicians and Surgeons, Columbia University, and Goldwater Memorial Hospital, New York, N. Y.

9:00 A.M. –

Greetings from the Academy – Albert S. Gordon, Chairman, Section of Biology, The New York Academy of Sciences, New York, N. Y.; New York University, New York, N. Y.

Introduction to Conference – Abraham Dury, Dorn Laboratory for Medical Research. Bradford Hospital, Bradford, Pa.

"Biogenesis of Phosphatides and Triglycerides" – Roger J. Rossiter and K. P. Strickland, Faculty of Medicine, University of Western Ontario, London, Ont., Canada.

"Recent Aspects of Cholesterol Biosynthesis and Catabolism" – Ezra Staple, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

"The Mechanism of Cholesterol Absorption" – Leon Swell, E. C. Trout, Jr., J. R. Hopper, H. Field, Jr., and C. R. Treadwell, General Medical Research, Veterans Administration Center, Martinsburg, W. Va., and The George Washington University, Washington, D. C.

"Structure and Homogeneity of Low-Density Human Serum Lipoproteins" – Frank T. Lindgren, Donner Laboratory, University of California, Berkeley, Calif.

"Chylomicrons and Lipid Transport" – Joseph H. Bragdon, Section on Metabolism, National Heart Institute, Public Health Service, Bethesda, Md.

Discussion Leaders: Robert G. Langdon, Johns Hopkins School of Medicine, Baltimore, Md.
Carleton R. Treadwell, The George Washington University School of Medicine, Washington, D. C.

PITUITARY AND ADRENAL HORMONES – LIPIDS AND ARTERIOSCLEROSIS

Session Chairman: Cecil Entenman

United States Naval Radiological Defense Laboratory, San Francisco, Calif.

2:00 P.M. –

"Some Hormonal Influences on Experimental Cardiovascular Disease" – Ernesto D. Salgado, Pfizer Therapeutic Institute, Maywood, N. J.

"Growth Hormone and Cardiovascular Changes in Choline-Deficient Rats" – George F. Wilgram, Banting and Best Medical Research Institute, University of Toronto, Toronto, Canada.

"Influence of Cortisone on Lipid Distribution and Atherogenesis" – Abraham Dury, Dorn Laboratory for Medical Research, Bradford Hospital, Bradford, Pa.

"Adrenal Medullary Hormones and Arteriosclerosis" – Yvo Thomas Oester, Department of Pharmacology and Therapeutics, Loyola University Medical School, Chicago, Ill.

Discussion Leader: Robert W. Wissler, University of Chicago, Chicago, Ill.

SATURDAY, APRIL 12, 1958

GONADAL, THYROID, AND PANCREATIC HORMONES – LIPIDS AND ARTERIOSCLEROSIS

Session Chairman: Alfred Steiner

Research Service, Goldwater Memorial Hospital, New York, N. Y.

9:00 A.M. –

"Causative Relationships of Parathyroid Hormone to Renogenic and Reniprival Cardiovascular Disease" – David Lehr, New York Medical College, New York, N. Y.

"Effects of Sex Steroids on Lipids" – Roger W. Robinson, Norio Higano, and William Cohen, Memorial Hospital, Worcester, Mass.

"The Serum Lipid Pattern in Hyperthyroidism, Hypothyroidism, and Coronary Arteriosclerosis" – Richard J. Jones, Louis Cohen, and Howard Corbus, Department of Medicine, University of Chicago, Chicago, Ill.

"The Pancreas and Cardiovascular Disease"—Charles W. Mushett, Research Laboratories, Merck Sharp & Dohme, Rahway, N. J.

Discussion Leaders: Simon Rodbard, Chronic Disease Research Institute, University of Buffalo, Buffalo, N. Y.
Howard A. Eder, Albert Einstein College of Medicine, New York, N. Y.

ENDOCRINES AND GROUND SUBSTANCE

Session Chairman: Carl Meyer

College of Physicians and Surgeons, Columbia University, New York, N. Y.

2:00 P.M.—

"Enzyme Activities of Human Arterial Tissue"—John E. Kirk, Division of Gerontology, Washington University School of Medicine, St. Louis, Mo.

"Effects of Tissue Age and Sex on Connective Tissue Metabolism"—Robert J. Boucek, Nancy Lee Noble, and J. Frederick Woessner, Jr., Howard Hughes Medical Institute, Miami, Fla.

"Hormonal Control of Permeability and Mobilization of Fat Depots"—Joseph Seifter and David H. Baeder, Wyeth Institute for Medical Research, Philadelphia, Pa.

"Effects of Hormones on Connective Tissue and Mucoproteins"—Norman F. Boas, Research Division, Norwalk Hospital, Norwalk, Conn.

SUMMARY DISCUSSION OF CONFERENCE

Chairmen of Sessions: Forrest E. Kendall
Cecil Entenman
Alfred Steiner
Carl Meyer

NEW MEMBERS

Elected April 24, 1958

LIFE MEMBERSHIP

Turner, Horace Edward, M.D., Orthopedic Surgery. Private Practice, Evanston, Ill.

SUSTAINING MEMBERSHIP

Barinbaum, Gisela S., Ph.D., Psychoanalysis. Consultant, Family Service Association, Woodmere, L. I., N. Y.

Burr, Charles Gilbert, M.D., Bone, Psychology. Physician, Surgeon, Private Practice, Houlton, Me.

Dooley, Francis M., M.D., Medicine, Oncology. Chief, Obstetrics-Gynecology, Mercy Hospital, Portland, Me.

Foe, Richard B., M.D., Internal Medicine. Physician, Private Practice, Greeley, Colo.

Gardner, David A., D.O., Internal Medicine. Active Practice as Internist, Ferguson, Mo.

Graybill, M. B., M.D., Surgery. Surgeon, Graybill Clinic, Escondido, Calif.

Hurvitz, Martin, M.D., Psychiatry. Private Practice, Roslyn Heights, N. Y.

Kling, Ralph André, M.D., Internal Medicine. Assistant Professor, New York Medical College, New York, N. Y.

Nelson, Richard John, M.D., Internal Medicine. Clinical Instructor, Department of Medicine, University of Utah College of Medicine, Salt Lake City, Utah.

Overton, Jane H., Ph.D., Problems of Growth and Development. Assistant Professor, Hull Zoological Laboratory, University of Chicago, Chicago, Ill.

Pearlmutter, Samuel B., M.D., Chemistry. Private Practice, Revere, Mass.

Reid, Robert W., M.D., General Surgery. Private Practice, Key West, Fla.

Wolberg, Arlene, M.S.S., Psychology. Director, Public Education Department, Post Graduate Center for Psychotherapy, New York, N. Y.

Young, David G., Jr., M.D., Physical Medicine and Rehabilitation. Observing Resident Physician, Hospital of University of Pennsylvania, Philadelphia, Pa.

Zilboorg, Gregory, M.D., Psychiatry, Psychology. Clinical Professor, Psychiatry, New York Medical College, New York, N. Y.

ACTIVE MEMBERSHIP

Adelman, Barnet R., M.S., Propulsion Systems for Rockets. Manager, Solid Propellant Rocket Engines Department, Ramo-Wooldridge Corporation, Los Angeles, Calif.

Albrecht, George H., M.S., Chemistry. Chemical Engineer, Operations Research Group, Army Chemical Center, Md.

Alcorn, W. J., M.D., Medicine. Physician, Surgeon, Private Practice, Wabasso, Minn.

Allred, William B., B.Ch.E., Nuclear Engineering. Manager, Engineering Development and Test, Combustion Engineering, Inc., Windsor, Conn.

Andelman, S. Y., M.D., Medicine. Physician, Private Practice, Tulsa, Okla.

Andrews, Thomas G., Ph.D., Psychology. Professor, University of Maryland, College Park, Md.

Angyal, Alice F., Ph.D., Psychology. Psychotherapist, Private Practice, Boston, Mass.

Aptekar, Herbert H., D.S.W., Social Work. Executive Director, Jewish Community Services of Long Island, Long Island, N. Y.

Arai, Tadashi, M.D., Antibiotic Research. Visiting Fellow, University of Pennsylvania School of Medicine, Philadelphia, Pa.

Arnold, Magda B., Ph.D., Psychology. Professor, Loyola University, Chicago, Ill.

Arnone, Peter R., M.D., Endocrinology. Clinical Assistant, Beth David Hospital, Brooklyn, N. Y.
Arnone, Rosario, M.D., Medicine. General Practice, Brooklyn, N. Y.
Bajek, Eleanore M., B.A., Pharmacology-Toxicology. Research Assistant, Garfield, N. J.
Baker, R. C., Ph.D,, Food Technology. Associate Professor, Poultry Husbandry, Cornell University, Ithaca, N. Y.
Baker, Robert Lee, M.D., Gynecology. Gynecologist, Private Practice, Salisbury, Md.
Ballou, James Monroe, M.D., General Surgery. Chief of Staff, Elliot Community Hospital, Keene, N. H.
Barahona, Juan Concha, M.D., Neurophysiology. Professor, Physiology, Concepción Medical School, Concepción, Chile.
Barger, William Calvin, M.D., Psychology. School Psychiatrist, Bureau of Child Guidance, Board of Education, New York, N. Y.
Bartlett, Neil R., Ph.D., Psychology. Professor, Hobart & William Smith Colleges, Geneva, N. Y.
Baum, Walter R., Ph.D., Mathematics. Associate Professor, Syracuse University, Syracuse, N. Y.
Beach, Edward W., M.D.,Urologic Surgery. Urologist, Mercy Hospital, Sacramento, Calif.
Bembry, Thomas H., Ph.D., Natural Products. Professor, Chemistry, City College of New York, New York, N. Y.
Berger, J. Joel, B.S., Chemistry. Teacher, High School Chemistry, Brooklyn, N. Y.
Bieber, Theodore I., Ph.D., Chemistry. Associate Professor, University of Mississippi, University, Miss.
Billingham, Rupert Everett, D.Sc., Tissue Transplantation Immunity. Professor, Zoology, University of Pennsylvania, Philadelphia, Pa.
Bojalil, Luis F., M.D., Mycobacteria. Chief, Department of Bacteriology, Uni-Mexico, D.F., Mexico.
Borysko, Emil, Ph.D., Biology-Cytology-Electron Microscopy. Research Associate, Institute for Dental Research, New York, N. Y.
Boscoe, Carmen Thomas, M.D., Cardiology, Physician, Surgeon, Private Practice, Oakland, Calif.
Bouillette, Pierre, E.P.C.I., Aromatic Chemicals and Specialties. Technical Advisor, Coordinator, The Givaudan Corporation, New York, N. Y.
Bower, John R., M.D., Hypnosis, Psychiatry. Private Practice, Reading, Pa.
Brennan, David Michael, Ph.D., Endocrinology, Reproductive Physiology, Assistant Professor, Biology, University of Notre Dame, Notre Dame, Ind.
Brennan, Francis T., B.S., Pharmacology. Pharmacologist, Smith, Kline & French Laboratories, Philadelphia, Pa.
Browne, Freeman, M.D., Cytology. Practicing Physician, Obstetrics-Gynecology, Oakland, Calif.
Capeci, Nicholas E,, M.D., Pharmacology. Staff Physician, Merck, Sharp & Dohme, West Point, Pa.
Carnow, Bertram Warren, M.D., Cardiopulmonary Physiology. Member, Department of Chest Diseases, Michael Reese Hospital, Chicago, Ill.
Carthey, Frank J., M.D., Medicine. Physician, Private Practice, New Ulm, Minn.
Cawthon, Thomas M., Ph.D., Physical Chemistry. Project Leader, Allied Chemical-Central Research Laboratory, Morristown, N. J.
Cedars, Nathan, M.D., Cancer Chemotherapy. Assistant Surgeon, Stephenville Hospital and Clinic, Stephenville, Texas.
Chappen, Edward Peter, M.D., Medicine, General Practitioner, Trenton, N. J.
Clevans, Ethel, M.S., Psychoanalytic Psychology. Private Practice, New York, N. Y.
Clum, Harold H., Ph.D., Plant Physiology. Professor, Hunter College, New York, N. Y.
Coates, Helen V., D.V.M., Experimental Pathology. Graduate Student, Johns Hopkins University, Baltimore, Md.
Corbett, John J., Ph.D., Protozoology. Research Professor, Biology, Manhattan College, New York, N. Y.
Constant, George A., M.D., Psychiatry, Neurology. Private Practice, Victoria, Texas.

Corso, Philip F., M.D., Tissue Homotransplantation. Chief Resident, Plastic Surgery, Bronx Veterans Administration Hospital, Bronx, N. Y.

Cotter, William Bryan, Jr., Ph.D., Genetics, Evolution. Assistant Professor, Biology, College of Charleston, Charleston, S. C.

Criep, Leo H., M.D., Allergy, Hypersensitivity. Associate Professor, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

De Fede, Anton J., M.D., Pediatrics. Private Practice, Bakersfield, Calif.

De Forest, Robert E., M.D., Cardiopulmonary. Physiology Section, Aeromedical Acceleration Laboratory, U. S. Navy Development Center, Johnsville, Pa.

Deiches, Edythe, M.S.S.W., Mental Health. Chief Psychiatric Social Worker, Middlesex County Mental Health Clinic, New Brunswick, N. J.

de Milies, Rosa G., M.D., Leukemia. Research Visiting Fellow, Sloan-Kettering Institute, New York, N. Y.

Derrick, C. Jennings, M.D., Pediatrics. Pediatrician, West Palm Beach, Fla.

Deutsch, Joel, M.D., Medicine. Private Practice, General Surgery, Philadelphia, Pa.

Dinerstein, Robert A., M.S., Petroleum Refining. Section Leader, Research Department, Standard Oil Company (Indiana), Whiting, Ind.

Ditter, Jerome F., Ph.D., Boron Hydrides Research. Supervisor, Physical Chemistry Section, Olin Mathieson Corporation, Pasadena, Calif.

Dixon, Stuart E., Ph.D., Insect Physiology, Biochemistry. Assistant Professor, Ontario Agricultural College, Guelph, Ontario, Canada.

Douglass, Frederick M., M.D., Surgery, Surgical Physiology. Private Practice, Toledo, Ohio.

Dreguss, Miklos N., M.D., Virology, Immunology. Staff Member, Division of Biologics Standards, National Institutes of Health, Bethesda, Md.

Dworecki, Izaak J., M.D., Medical Bacteriology. Assistant Pathologist, St. Clare's Hospital, New York, N. Y.

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Eberstein, Igor John, Nuclear Science, Rocketry. Student, Cooper Union, New York, N. Y.

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Eisdorfer, Carl, M.A., Psychology. Assistant, Medical Psychology, Duke University School of Medicine, Durham, N. C.

Eisenstein, T. Donald, M.D., Pediatrics. Assistant Resident, Pediatrics, New York, N. Y.

Englander, Jacob A., D.D.S., Dentistry. Assistant Professor, Endodontia, New York University College of Dentistry, New York, N. Y.

Evers, Ray, M.D., Medicine. Physician, Surgeon, Andalusia, Ala.

Feldman, Joseph Aaron, Ph.D., Analytical Pharmacy. Assistant Professor, Pharmaceutical Chemistry, Duquesne University School of Pharmacy, Pittsburgh, Pa.

Feldman, Yonata, M.A., Psychology. Casework Supervisor, Madeline Borg Child Guidance Institute, New York, N. Y.

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Johnstone, Mary C., B.Sc., Biology. Assistant Editor, Medical Science, New York, N. Y.

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Jones, William Ralph, B.S., Mercurial Diuretics. Technician, Pharmacology, Pitman Moore Company, Indianapolis, Ind.

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Kissin, Benjamin, M.D., Psychosamatic Medicine, Director, Alcoholism Research Clinic, New York State University College of Medicine, Brooklyn, N. Y.

Klimt, Christian, R., M.D., Public Health. Epidemiologist, W.H.O., Bilharzia Control Project, Baghdad, Iraq.

Knutsson, Katherine H., M.D., Psychiatry. Private Practice, Palo Alto, Calif.

Kornegay, Grey Bryan, Sr., M.D., Medicine. Physician, Private Practice, Wallace, N. C.

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Kuno, Yas, M.D., Physiology. Professor Emeritus, Nagoya University, Kyoto, Japan.

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Levin, Jack J., M.D., Internal Medicine. Assistant Chief, Medical Service Veterans Administration Center, Wood, Wis.

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Lipowitz, Melvin W., B.A., Biological Sciences. Technician, Clinical Chemistry, Newark Beth Israel Hospital, Newark, N. J.

Loo, Edward C., M.D., Mycology. Chief, Medical Service, Harford Memorial Hospital, Havre de Grace, Md.

Looze, Joseph P., M.D., Biology. Staff Physician, Veterans Administration Hospital, Wood, Wis.

Louw, Joan, D.Sc., Medical, Premedical Sciences. Research Assistant, National Research Center for Science, Paris, France.

Lowrance, Preston B., M.D., Physiology, Internal Medicine. Assistant Professor, University of Virginia School of Medicine. Charlottesville, Va.

Lowry, Houston, M.D., Medicine. Private Practice, Madisonville, Tenn.

Lukas, Daniel S., M.D., Cardiac, Pulmonary. Physiology. Associate Professor, Medicine, Cornell University Medical College, New York, N. Y.

Luke, Edward A., M.D., Medicine. General Practitioner, Fort Worth, Texas.

McCrumb, Fred Rodgers, Jr., M.D., Infectious Diseases. Assistant Professor, Medicine, University of Maryland School of Medicine, Baltimore, Md.

McDermott, Philip S., M.S., Physical Chemistry. Chemist, Rohm & Haas Company, Philadelphia, Pa.

McGinniss, Gerald F., M.D., Neurophysiology. Research Associate, Brookhaven National Laboratory, Upton, L. I., N. Y.

Malin, Sarah Arpe, M.D., Medicine. Physician, Walnut Creek, Calif.

Manso, Carlos Filipe de Aguilar, M.D., Biology. Sloan Research Fellow, Sloan-Kettering Institute for Cancer Research, New York, N. Y.

Marks, Donald Elliott, M.A., Physiology. Research Technician, Walter Reed Army Medical Center, Washington, D. C.

Medinets, Howard E., M.D., Neurological Surgery. Private Practice, South Orange, N. J.

Millen, Robert S., M.D., Obstetrics-Gynecology. Chief, Obstetrics-Gynecology, The Community Hospital, Glen Cove, L. I., N. Y.

Minor, Henry W., M.D., Medicine. Physician, Private Practice, Atlanta, Ga.

Mishra, R. K., M.B., Experimental Medicine. First Research Associate, Institute of Medicine and Experimental Surgery, University of Montreal, Montreal, Canada.

Mizer, F. Robert, M.D., Internal Medicine. Physician, Private Practice, Denver, Colo.

Moody, Rollen Wayne, M.D., Medicine. Private Practice, Denver, Colo.

Morcy, Madeleine E., M.D., Pediatrics. Regional Medical Director, U. S. Children's Bureau, Charlottesville, Va.

Moses, Arthur, M.D., Viruses, President, Brazilian Academy of Sciences, Rio de Janeiro, Brazil.

Moss, John A., M.D., General Surgery. Private Practice, Barberton, Ohio.

Moyle, Henry B., M.B., Anthropology, Psychology. Psychiatric Consultant, State Home for Children, Litchfield, Conn.

Neidig, Howard A., Ph.D., Physical, Organic Chemistry. Chairman, Department of Chemistry, Lebanon Valley College, Annville, Pa.

Newman, Ben A., M.D., Biology. Chairman, Department of Dermatology, Cedars of Lebanon Hospital, Los Angeles, Calif.

Olsen, Kaj, M.D., Cancer Research, Virology. Research Fellow, University of California, Berkeley, Calif.

Pensig, Arnold W., M.D., Internal Medicine. Medical Department, Pfizer Laboratories, Brooklyn, N. Y.

Peters, Hans J., M.D., Neuropathology. Instructor, Pathology, Medical College of Georgia, Augusta, Ga.

Pettit, Mary DeWitt, M.D., Gynecology-Obstetrics. Professor, Woman's Medical College of Pennsylvania, Philadelphia, Pa.

Pfeffer, Peter, Electronics. Engineer, Bendix Aviation Corporation, New York, N. Y.

Pistey, Warren R., M.D., Parasitology. Post-Doctoral Fellow, New England Institute for Medical Research, Ridgefield, Conn.

Pollock, John J., Jr., B.S., Pharmacology. Junior Pharmacologist, Philadelphia, Pa.

Potters, Milton, D.D.S., Periodontal Membrane. Periodontist, Private Practice, Jersey City, N. J.

Powsner, Edward R., M.D., Hematology, Fellow, Wayne State University College of Medicine, Detroit, Mich.

Rath, Charles K., M.D., General Surgery, Multiple Malignancy. Surgeon, Veterans Administration Hospital, Beckley, W. Va.

Razdow, Adolph, Ph.D., Color Film Television, Optical Gaging. Vice-President, Bryg Inc., Montclair, N. J.

Rintel, Leon, M.A., Science Education. Chairman, Biology Department, Forest Hills High School, Forest Hills, N. Y.

Rizzo, Anthony A., D.M.D., Dental Research. Principal Investigator, National Institute of Dental Research, Bethesda, Md.

Robinson, Guy U., M.D., Internal Medicine. Private Practice, Dumas, Ark.

Rogawski, Alexander S., M.D., Psychoanalysis. Instructor, Psychiatry, University of Southern California, Los Angeles, Calif.

Rose, Lawrence R., M.D., Plastic Surgery, Fellow in Plastic Surgery, Brentwood, Mo.

Rosenberg, Norman, Ph.D., Analytical Chemistry. Research Chemist, Air Force Laboratory, Newton Centre, Mass.

Rosenblum, Lee Allen, M.D., Medicine. Pathologist, Chief of Laboratories, 121st Evacuation Hospital, APO 20, San Francisco, Calif.

Rubin, Albert L., M.D., Cardiovascular-Renal Diseases. Assistant Professor, Medicine, Second (Cornell) Medical Division, Bellevue Hospital, New York, N. Y.

Sanchez, Alex F., M.D., Cytology of Genetical Tract, General Practitioner, Plant City, Fla.

Saracino, René, M.D., Cancer. Head, Clinicotherapeutic Department, Rene Huguensis Anti-Cancer Center, St. Cloud, France.

Scheer, Milton D., Ph.D., Physical Chemistry, Physical Chemistry Specialist, General Electric Company, Silver Spring, Md.

Schlossberg, Harvey, B.S., Psychology. Student, Long Island University, Brooklyn, N. Y.

Schulman, Martin P., Ph.D., Biochemistry. Assistant Professor, State University of New York, College of Medicine, Syracuse, N. Y.

Schweid, Abraham I., M.D., Pathology, Assistant Laboratory Officer, U. S. Naval Hospital, Great Lakes, Ill.

Seibold, Herman R., V.M.D., Veterinary Pathology. Veterinarian-Pathologist, Plum Island Animal Disease Laboratory, Greenport, L. I., N. Y.

Selke, Oscar O., Jr., M.D., Physical Medicine and Rehabilitation. Physician, Hermann Hospital, Houston, Texas.

Shanbrom, Edward, M.D., Hematology. Chief, Leukemia-Lymphoma Section, City of Hope Medical Center, Duarte, Calif.

Ship, Irwin I., D.M.D., Dental Medicine. Principal Investigator, Clinical Investigations, National Institute of Dental Research, Bethesda, Md.

Shmukler, Herman William, M.S., Biochemistry, Biochemist, Naval Air Development Center, Johnsville, Pa,

Slavson, S. R., B.S., Group Psychotherapy. Consultant, New York, N. Y.

Sloboda, Adolph E., Cancer. Biologist, American Cyanamid Company, Pearl River, N. Y.

Smart, Reginald Hughes, M.D., Pulmonary Diseases, Cardiorespiratory Physiology. Clinical Professor, Medicine, University of Southern California School of Medicine, Los Angeles, Calif.

Smith, Andrew George, Ph.D., Bacterial Cytology. Associate Professor, Microbiology, University of Maryland, School of Medicine, Baltimore, Md.

Smith, Bianca, M.D., Surgery. Chief Surgical Resident, Santa Barbara General Hospital, Santa Barbara, Calif.

Solano, Sister Francis, Ph.D., Biology. Professor, Nazareth College, Rochester, N. Y.

Sprengling, Gerhard Roy, Ph.D., Chemistry. Section Manager, Plastics, Westinghouse Research Laboratories, Pittsburgh, Pa.

Stahl, Robert R., M.D., Hypertensive Vascular Disease. Instructor, Medicine, Western Reserve University, Cleveland, Ohio.

Stasior, Orkan George, M.D., Ophthalmology. Resident in Ophthalmology, New York, N. Y.

Stephens, John W., M.D., Diabetes, Internal Medicine. Clinical Instructor, University of Oregon Medical School, Portland Oreg.

Stevenson, Peter Cooper, Ph.D., Radiochemistry, Nuclear Fission. Research Radiochemist, University of California Radiation Laboratory, Livermore, Calif.

Strauss, Alvin W., Jr., M.D., Medicine. Private Practice, Little Rock, Ark.

Strong, Edward K., M.D., General Surgery. Staff Surgeon, Southern Pacific General Hospital, San Francisco, Calif.

Taleghany, Parviz, M.D., Chemistry, Biology. Department of Surgery, Memorial Center for Cancer and Allied Diseases, New York, N. Y.

Tasch, Paul, Ph.D., Invertebrate Paleontology. Associate Professor, Geology, University of Wichita, Wichita, Kans.

Tasker, Samuel, M.D., Dermatology. Private Practice, Los Angeles, Calif.

Teillon, Jean, D.Sc., Microbiology. Head, Bacteriology Department, Antibiotic Fabrication Industrial Society, Paris, France.
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